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ON THE BIOLOGICAL FACTORS INVOLVED IN THE DESTRUCTION OF RIVER-FISHERIES BY POLLUTION DUE TO LEAD-MINING

BY KATHLEEN E. CARPENTER, M.Sc.
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A PREVIOUS article (1) has recounted a series of observations carried on in the Aberystwyth district of Cardiganshire between the years 1919 and 1923, as a consequence of which the writer was led to the belief that impoverishment of the fauna of lead-polluted streams is associated rather with the presence of metallic salts dissolved in the water than with that of galena-grit. The latter substance occurs in quantity in the beds of practically all streams of the district under observation, especially in that of the Clarach, which contains trout, sewen, eels and sticklebacks, as well as a rich Invertebrate fauna; metallic solutions, on the other hand, have been found to occur only in waters which have suffered fresh pollution from recent lead-mining and washing operations—notably in those of the Rivers Rheidol and Ystwyth, which for a number of years have been thus rendered absolutely barren of fish-life and extremely poor in general fauna and flora. The cessation of works affecting these rivers by 1921 was followed by a partial restoration of the fauna and flora, and since that time, although galena-grit is still present in their beds, the dissolved lead-content of the waters has fallen to *nil* at ordinary times, though in times of flood the supply is partially renewed, probably owing to chemical action of surface-waters over the dump-heaps. Since 1922, however, the quantity in the Rheidol has never risen beyond the equivalent of 0·5 milligramme of the lead base to 1 litre of water, and a very exceptional flood is required to bring the lead into solution at all. The Ystwyth has suffered a fresh pollution of a rather new character, owing to the establishment of zinc-dressing works on its bank, but the improvement in the Rheidol has continued without a check since 1921, and by summer 1923, representatives of all the usual groups of aquatic animals, with the exception of Mollusca and Fishes, were to be found in its waters.

It was decided at this time to undertake a series of experiments upon Fishes themselves in relation to the polluted waters. The issue was by no means clear at the start: although circumstantial evidence had convinced the writer that their absence from these waters was in some way related to the presence of lead in solution, it was difficult to suppose that such low concentrations could be directly efficacious in this connection. Alternative theories suggested themselves: the one, that dilute solutions might act directly as a cumulative poison upon the fish-organism—the other, that the absence of fishes was due simply to a lack of available food material, and referable ultimately to the effect of these dilute solutions upon aquatic plants and possibly also upon Invertebrates. Neither theory adequately covered the situation of the moment, since at the time the plant- and Invertebrate-population were well on the way towards normality, yet no trout had come in from the tributary streams to feed on them, while on the other hand, the occurrence of lead in solution was now only intermittent, precluding the possibility of cumulative action since 1922. The practical difficulties in the way of putting either theory directly to test were considerable, but, by way of an initial casting of bread upon the waters, it seemed worth while to ascertain whether fishes could exist for any length of time, and if so for how long, in the now intermittently polluted stream.

A spot was carefully chosen in the Lower Rheidol, at Pen-y-Geulan, about seven miles above the sea, in non-tidal water, and a fish-cage (lent by the Ministry of Agriculture and Fisheries) moored to a stake on the convex curve of the river-bank in such a way as to float in full stream over water several feet in depth. Minnows (*Leuciscus phoxinus*) were chosen as the subjects of experiment, as being hardy fish, and fairly easily obtained in the district, and six of these fish were placed in the cage on August 31st, 1923. Galena-containing grit from the river-bed (a sample specimen of which contained lead to the extent of 0·49 per cent. of the dry weight) was kept on the floor of the cage, and any suspended matter in the river water, as well as substances in solution, passed continually through the perforated zinc which formed the walls: the fish were thus exposed as far as possible to all the characteristic conditions of the river. At the commencement of the experiment, the water was only moderately high, and the lead-content, as ascertained by analysis, *nil*. Between this time and October there was no rise in the water-level, and the fishes remained living and apparently healthy. October was a month of heavy rainfall: by the 23rd the river was in flood, three fishes were dead, and the water-sample showed lead to be

present in solution, as usual during a flood. Unfortunately, it was necessary to conclude the observations on this day, and the experiment remained incomplete. It is reported in full only because it furnished data for the drawing-up of a scheme of future work, which data may be summarised as follows:

(*a*) The fishes lived for several weeks in the river during a period of calm: hence the water in its normal state was probably not harmful to them;

(*b*) The coincidence of the death of three out of six individuals with the onset of flood-conditions appeared significant;

(*c*) The only post-mortem symptom observed was the presence of an unusually large quantity of thick mucus around the bodies; no trace of grit or fine sediment was found adhering to the gills, nor was there any bruising; death appeared thus to be related to the change in quality of the river-water itself, a change marked by the solution of lead.

While an experiment so incomplete could not be made the basis of final conclusions, the above data were felt to be of sufficient importance to justify the undertaking of a continuous series of experiments, using a large number of fishes. The scheme adopted aimed to determine as accurately as possible, by repeated experiments in the field, the conditions and symptoms accompanying the death of Fishes in the polluted stream; next, by selective use of river-waters, grits and sludges in the laboratory, to fix upon the essential and responsible factors; finally, to reconstruct the conditions by artificial methods so accurately that fishes should be killed in the requisite time and with the characteristic symptoms. It was felt that not until this final clause had been satisfied could the actual lethal factor or factors be designated with certainty.

In the field-experiments, the same simple method was followed throughout: fishes were placed in the cage, exposed as far as possible to the natural conditions prevailing in the river, and kept there until they died: they were then removed and subjected to careful post-mortem examination. Samples of the water were tested for lead at the commencement and end of the experiment, and at intervals of a few days during its course, though experience had shown that this was scarcely necessary, lead only coming into solution when the water reached flood-level.

In the second experiment, seven minnows were put into the cage on November 6th, the water being fairly high, but without lead in measurable quantity. On November the 9th, the conditions were unaltered: heavy rain fell during the next 24 hours (rainfall records for correlation

with the field-work are appended at the end of this article), and on November 10th, all seven minnows were dead. The water sample contained between 0·3 and 0·4 milligramme of lead per litre. Post-mortem examination of the bodies revealed no other symptom than the coagulation of mucus into a thin gelatinous film over the whole body and a thicker coating over the gill-filaments, which entirely disguised their normal reddish colour.

A sample of the flood-water obtained on November 10th was filtered, and two minnows were placed in the filtrate in the laboratory: both died in less than sixteen hours (actually during the night), with the symptoms described above. The control animals, in normal river-water (not at flood), were kept under observation for a week.

The responsibility for death in the case of minnows was thus definitely fixed upon the dissolved content of the flood-water, as differing from that of normal times; the author's alternative theories of cumulative action and of starvation from lack of food-material were entirely discredited, and the process, though not yet thoroughly understood, was seen to be rapid and direct, involving the coagulation of mucus as an attendant circumstance.

In January, 1924, by the kindness of the Aberystwyth Anglers' Association, a few yearling trout were obtained alive and in good condition, and two of these were placed in the cage on January 18th; the water was at a moderate level, and contained no trace of lead. Both were living on the 21st; on the following morning, after some rain had fallen, the water contained a minute trace (impossible to estimate accurately) of dissolved lead, and one fish was dead. The other survived, and on February 4th a third was added to it, the lead-content having fallen to *nil*, where it remained during two months of comparative drought. Between March 22nd and 24th, heavy rains fell; on the 24th, at 11 a.m., the lead-content was 0·4 milligramme per litre, and one fish was already dead: the other expired a few hours later. The typical film of coagulated mucus was exhibited in each case. While the death of the first trout may possibly be regarded as accidental, yet it should be noted that it was coincident with the appearance of a minute quantity of lead in solution—a quantity, however, which was not sufficient or was not maintained for a long enough time to kill the second. Leaving this point aside, the survival of the two fishes through a protracted period and their practically simultaneous death, with the familiar symptoms, immediately after the flooding of the river, gives rise to conclusions similar to those drawn from experiments upon minnows. These conclusions were

further borne out by the death (in 8–21 hours) of a single trout placed in a filtrate of the flood-water in the laboratory, and the survival of a fifth which was placed in water taken from the river during the drought and free from lead.

Another field-experiment using minnows was commenced on June 1st, 1924, six fish being placed in the cage. The river was at this time fairly high, but there was no dissolved lead-content, nor has any sample taken since that date contained a trace of lead in this form, although there has been no shortage of rain. Up to the time of writing (October 8th, 1924) the fish are still alive and apparently healthy. The fact is interesting in connection with the natural establishment of sticklebacks (*Gastrosteus aculeatus*) in the Lower Rheidol which has at last taken place this summer: it would seem that the river is in a fair way to complete rehabilitation, the process of intermittent pollution from the dumps being at any rate in abeyance, probably owing to the washing-out of material from their riverward slopes. Whether the improvement will be permanent it is impossible to say: the dumps are very extensive, and weathering may at any time expose a fresh supply of material to the action of surface waters. Meanwhile the experiment goes on, and serves to illustrate the possibility of survival for a protracted period in the absence of soluble lead.

The net result of the field-experiments and of all laboratory experiments with river-waters is to stress the importance of the chemical alteration of the water at flood-times as an agent in the destruction of fishes—a conclusion distinctly subversive of the current theory, which ascribes the death of fishes in lead-mining areas to the clogging of their gills by adhering particles of galena-grit, and according to which damage to fisheries can best be avoided by protecting dump-heaps and by running the mine-effluents through settling-pits, so as to keep solid particles out of the river. A theory which, on grounds of probability, has met with such general acceptance, and which bears such important practical consequences, could not be lightly dismissed in the course of this inquiry, even though no case of clogging by solid particles had ever been met with among the fishes exposed to such a risk in the cage. An attempt was therefore made to test it directly in the laboratory. Finely divided material from a mine-dump at Goginan, containing galena-particles (lead, 1·64 per cent. of the dry weight), was placed in a glass tank to cover the bottom to a depth of half an inch, water from the minnow pool was introduced to a total depth of one and a half inches, and three minnows were placed therein. The movements of the fish rendered the

water continually turbid, but on examination after six days the gills were found to be perfectly clean and the general condition normal. A second experiment of this character was maintained during six weeks, with frequent aëration of the water, and had a like result. Any theory of the efficacy of lead-grit in this connection must therefore be abandoned; nor does the semi-colloidal slime which accumulates in the later settling-pits of an active mine appear to be necessarily harmful, since a further experiment made in July, 1924, using material of this character obtained from a mine near the Teifi, also gave a negative result.

Sole responsibility was thus finally fixed upon the dissolved content of the flood-water, the index to its fatal character being the presence of soluble lead: whether the lead itself, or some other accompanying factor, were the actual lethal agent had yet to be determined with certainty. The effects of dilute solutions of lead upon aquatic animals have been little studied: Drs Penny and Adams (2), using lead nitrate, have declared a solution of the concentration of 250 milligrammes per litre to be fatal to minnows and goldfish, and more recently, Dr W. Rushton (3) has found that a 10 mgr. solution of lead acetate will kill yearling trout, but these figures are very high, and moreover the published records in neither case bear any reference to control-experiments; it was felt to be desirable, therefore, to repeat the experiments with careful controls, before working down to the minimum concentration which should be fatal. In the series carried out, artificial solutions of lead nitrate, acetate and sulphate have been used in the test experiments, and in the controls distilled water from the same still as that used in making up the solutions, and at the same degree of hydrogen ion concentration—*p*H being 6·4 to 6·6, the normal figure for the river-waters (usually 6·4 in flood, about 6·6 at other times). A double control was also established using a natural water from the river (non-flood), or from the minnow pool. The possibility of experimental error was thus reduced to a minimum, and the whole weight of responsibility for any special result of the test-experiment was thrown upon the dissolved lead-salt. The advantages of the double-control system have been obvious: in an initial series of experiments made with controls in distilled water only, the whole of the fish, in tests and controls, succumbed—a fatality which could only be attributed to the presence of minute traces of copper derived from the still; the whole series had to be regarded as worthless and work recommenced, using water from a still of different type, which was proved to be innocuous. Similarly, the early intention of using tap-water as the "natural water" for the double control had also to be abandoned, owing to the highly

plumbo-solvent character of the local water. Since these experiences, only two of the control-animals have died—one by leaping out of the tank, the other from some unexplained cause—and the experiment is not in either case invalidated, owing to the double control.

An early series of experiments made in the autumn of 1923 confirmed the observations of the previous investigators, and established a lower figure—the equivalent of 5 mgr. of lead per litre—as being fatal to minnows. The post-mortem symptoms were in all cases those characteristic of death in the flood-waters, although in the case of strong solutions they were much exaggerated, the mucus layer becoming extremely thick. The reaction-time varied from $2\frac{1}{2}$ hours in the case of a 250 mgr. solution, to about 12 hours in that of a 5 mgr. solution. The latter concentration was tested in eight cases, using the acetate of lead, in four with the nitrate: there was no apparent difference in effect. A 5 mgr. solution of the nitrate was also used upon a yearling trout in January, with the usual fatal result.

The work with minnows was interrupted during the winter, owing to difficulties of supply, and some interim experiments were made upon goldfish (*Carassus auratus*), with interesting results. A 250 mgr. solution of nitrate proved fatal, indeed, but not until four days had elapsed—a great length of time as compared with the two or three hours required for the death of minnows. A saturated solution of lead sulphate (about 26 mgr. per litre) was fatal in about the same length of time, but a 10 mgr. solution of lead acetate produced no fatal effect during three weeks of observation. There are, it would seem, degrees of susceptibility among fishes to the action of lead solutions, goldfish being relatively very little susceptible, and thus, not suitable subjects for experiments with low concentrations. The value of the experiments already carried out, however, was considerable, for the slow action of the concentrated solutions afforded an opportunity for studying the effect on the animal in detail, as if in a "slow-motion picture." A goldfish placed in a strong lead solution began almost at once to show discomfort and spasmodic activity: by the end of 12 hours, the film of coagulated mucus was clearly visible, extending all over the body-surface, and up to the time of death the outer portions of this film continued to peel off in shreds, clouding the surrounding water. The film nevertheless grew thicker, by renewal from the body, and at death it was well-marked; but still more marked was the mass of apparently similar material which formed over the gill-filaments and began after a day or two to be visible below the edge of the operculum, whose aperture was in the end closed by it as if by a

packing of cotton-wool. During the last days the fish were unusually sluggish, floating almost motionless in the water, and the respiratory movements were extremely slow, feeble and intermittent. The whole circumstances suggested death by slow suffocation, with coagulation of mucus around the gills as the cause of death, and not a mere consequence or even an attendant circumstance. This theory was given further support by the behaviour of the goldfish which survived in a 10 mgr. lead solution; in this case, all the usual symptoms appeared during the first four days, and the animal seemed moribund, but after this time the thickened mucus which was shed off into the water was not renewed from the skin, and complete restoration to the normal condition followed. The facts suggested that the supply of lead in the given quantity of solution was not sufficient to complete the fatal process, and the 10 mgr. experiment was repeated, but this time with complete renewal of the whole quantity of solution on alternate days. The mucus-coating continued to accumulate, and death followed in the usual way in 13 days. The length of the period in the case of goldfish, and the quantity of solution required, may no doubt be referred to the general sluggish habit of these fishes, and the low rate of metabolism: life could be maintained, although at a very low ebb, even when respiration was in great part inhibited—a condition impossible to a fish of more active habit.

The nature of the apparently coagulated mucus was investigated by analysis, samples obtained from minnows which had died in artificial solutions and also in the natural flood-water being sent to the County Analyst, who found in them "considerable quantities" of lead in organic combination (probably in the form of the albuminate). Death of the fishes is thus ultimately referable to a chemical interaction between a secretion of the skin and the lead dissolved in the surrounding medium, producing a colloidal substance which when present in sufficient quantity inhibits respiration. This being the case, the actual quantity of lead available becomes important, and the success of the 10 mgr. experiment with goldfish in its revised form is readily understood.

Work with minnows was resumed in June, 1924, when the 5 mgr. experiment was repeated: death occurred in about 12 hours. A 1 mgr. solution of lead acetate was next employed, with a result exactly comparable with that obtained by using a 10 mgr. solution upon goldfish, only telescoped in time; development of the usual symptoms occurred within a very few hours, and by the following day recovery was complete, the mucus-coat being completely shed off. The fish remained normal during 13 days of observation, being kept in the original solution,

which was frequently aërated by means of a pipette. So far, the method of treatment for minnows had been to place each one in a vessel containing about 500 c.c. of the test-solution, and not to renew the latter, but to aërate it several times a day. (The fish used measured about 2–2¼ inches in length, this being the average size of sexually mature specimens collected locally.) In future experiments with dilute concentrations of lead salts, the whole quantity of liquid was renewed at intervals of a few hours, the water in the control experiments being, of course, renewed in exactly the same way and at corresponding times. In this way a 1 mgr. solution of lead acetate was found to be fatal to minnows after seven or eight renewals, a 1 mgr. solution of the nitrate in about the same time.

Solutions of lead of the concentration actually found in the poisonous flood-waters of the river (0·4 mgr. of lead per litre) were next employed, and the result was in all cases the same: whether the sulphate or the nitrate were employed, a fatal effect with all the characteristic symptoms (although the general coating of mucus in dilute solutions was relatively thin) ensued within 36 hours. The acetate was employed in seven cases, the nitrate in three.

The fatal effect of dissolved lead upon fishes is now seen to depend not primarily on the nature of the lead salt nor on the strength of the solution, but rather on the actual quantity of lead itself available. In a swiftly flowing stream the supply will be continually renewed, and even an extremely dilute solution, such as occurs intermittently in streams whose mines are no longer active, is quite sufficient for the extermination of the fishes. Under an active mine, the stronger solutions will have a correspondingly rapid effect.

It would seem not too difficult a matter to suggest a method of treating mine effluents which, by preventing the retention of this lead in solution, should render them innocuous—but the problem is not quite so simple.

Comparison of the results of experiments in the use of artificial solutions with those obtained from use of the flood-waters shows a marked discrepancy, not in the nature of the reaction but in the time-factor.

The time required for an artificial 0·4 mgr. lead solution to be fatal varies from 24 to 36 hours: the natural flood-waters are efficacious in a period not exceeding 12, and in this latter case the initial quantity of 500 c.c. is sufficient without renewal. The inference is that, while the quantity of lead in the flood-waters is quite sufficient to be efficacious, and while analysis of the coagulated mucus makes it clear that lead is a responsible agent, yet there must be present in the flood-waters some substance other than lead which acts as an accessory cause of death.

There is every indication that this substance is also of a metallic nature, and, like the lead, present only in minute quantities incapable of detection except by analysis especially directed to that end. A comparison of the flood-waters of Rheidol with those of Teifi and Meurig (neighbouring streams, rich in trout at the time of the comparison—December, 1923) shows a remarkable similarity in general character of the waters. No test of metallic content other than lead was applied (see Table C).

In the ore-deposits affecting the Rheidol, lead does not as a rule occur alone, but generally in association with zinc (as blende), occasionally with iron (as pyrites), and very rarely with copper. Of a total of 41 mine-workings, ancient and recent, affecting the Rheidol and its tributaries, zinc ore is reported from 27, lead from all, iron from 11, and copper from only three, none of which last are of recent date (4).

Of these metals, zinc is the only one which, in dilute solutions, produces an effect on fishes in any way comparable with that of lead, and preliminary experiments have shown that the effect of the flood-waters can be very well imitated, both in nature of symptoms and in time of reaction, by the use of a solution containing lead in the proportion of 0·4 mgr. per litre together with zinc at 0·8 mgr. per litre (the sulphate). Further analysis of the flood-waters is looked to, to furnish evidence of the occurrence of any of these metals other than lead, and experiments are now proceeding with a view to determining their relative biological importance. Such work alone can form the basis of a scheme for chemical treatment of the mine-waters, the responsibility for which must finally devolve upon the chemical expert. Meanwhile the biological evidence is clearly to the effect that the safety of river-fisheries is incompatible with the free entry into the rivers of mine-waters bearing lead (and in some cases other metals) in clear solution, and that no amount of sedimentation of these waters or of protection of dumps can be efficacious in preserving the fisheries so long as the clear water contains even very minute quantities of dissolved lead. Pending the determination of a method of chemical treatment, it may be possible in some districts to conduct the mine-outflow into a region where it can do no damage—*either* directly into the sea or some relatively enormous body of water where its effect will be lost, *or* into some local water which has already suffered pollution so damning that it has nothing more to lose. These are but tentative suggestions, dependent upon local conditions, and at best a mere temporising with a problem for whose ultimate solution much further investigation is required.

Table A.

Rainfall-records from Castle Point, Aberystwyth.

(It is, unfortunately, impossible to give, as in a previous article, records from Devil's Bridge, as observations at this station have been discontinued. The Castle Point records give only an approximate estimate of the distribution in time and quantity; the rainfall is much greater near the sources of the Rheidol.)

	August 1923	September 1923	October 1923	November 1923	December 1923	January 1924	February 1924	March 1924
1	0·11	0·05	0·09	0·28	0·12	0·16	—	0·16
2	0·02	—	0·30	0·19	0·04	0·05	—	0·06
3	—	0·43	0·52	0·04	0·22	0·15	—	0·44
4	—	—	0·06	—	—	—	—	—
5	0·02	—	0·14	0·42	0·01	—	—	0·32
6	0·03	0·08	—	0·02	0·10	—	—	—
7	0·02	—	0·34	—	—	0·05	0·02	—
8	0·02	—	0·59	0·69	0·01	—	—	—
9	0·21	0·01	0·05	0·04	—	0·02	0·06	—
10	—	—	0·50	—	—	0·12	0·03	—
11	—	0·04	0·03	0·01	0·07	0·52	—	—
12	—	0·04	0·20	0·40	—	0·26	—	—
13	0·03	0·33	0·46	1·12	0·02	0·39	—	—
14	0·05	0·21	0·13	0·08	—	—	—	—
15	—	0·43	0·04	0·09	—	—	0·08	—
16	0·19	0·09	—	0·20	0·02	0·08	—	—
17	0·03	0·70	0·09	0·01	—	0·05	0·30	—
18	0·02	0·05	0·15	0·03	0·09	0·22	—	—
19	0·11	0·32	—	0·44	0·03	0·10	—	—
20	0·12	0·22	0·25	0·02	0·08	0·25	—	—
21	0·20	0·23	0·53	—	0·14	0·32	0·02	0·03
22	—	0·24	0·20	—	0·22	0·10	—	0·57
23	0·34	0·09	0·35	—	0·08	0·08	—	0·28
24	—	0·10	0·48	—	0·44	0·02	0·12	—
25	0·43	0·17	0·32	—	0·43	—	—	0·02
26	0·03	—	0·46	—	0·23	0·12	—	—
27	0·03	0·16	0·29	—	0·10	—	0·03	—
28	0·12	0·06	—	—	0·22	0·15	—	—
29	0·35	0·03	0·09	—	0·04	—	0·16	—
30	0·15	—	0·14	0·04	0·18	0·03	—	—
31	0·29	—	—	—	—	0·04	—	—

Table B.

Experiments with Artificial Solutions of Lead Salts.

(The use of a double figure in column 4—*e.g.* 2–11 hours—indicates that death occurred during the night; exact time could not therefore be ascertained.)

B 1. Experiments performed on Minnows (*Leuciscus phoxinus*).

	(1) Nature of the lead salt	(2) Mgr. of lead per litre	(3) Treatment	(4) Period of observation	(5) Result
1	Nitrate	250	500 c.c. of solution not renewed	2–11 hours	Fatal
2	,,	250	,, ,, ,,	,, ,,	,,
3	,,	10	,, ,, ,,	,, ,,	,,
4	,,	10	,, ,, ,,	,, ,,	,,
5	,,	5	,, ,, ,,	,, ,,	,,
6	,,	5	,, ,, ,,	,, ,,	,,
7	Acetate	250	,, ,, ,,	2½ ,,	,,
8	,,	5	,, ,, ,,	8–23 ,,	,,
9	,,	5	,, ,, ,,	,, ,,	,,
10	,,	5	,, ,, ,,	8½–23 ,,	,,
11	,,	5	,, ,, ,,	,, ,,	,,
12	,,	5	,, ,, ,,	4–16 ,,	,,
13	,,	1	,, ,, ,,	13 days	Not fatal
14	,,	1	500 c.c. renewed 7 times	48 hours	Fatal
15	,,	1	,, ,, 8 ,,	53 ,,	,,
16	Nitrate	1	,, ,, 10 ,,	31 ,,	,,
17	,,	1	,, ,, 11 ,,	49 ,,	,,
18	Acetate	0·4	,, ,, 9 ,,	34–48 ,,	,,
19	,,	0·4	,, ,, 9 ,,	,, ,,	,,
20	,,	0·4	,, ,, 9 ,,	32–43 ,,	,,
21	,,	0·4	,, ,, 9 ,,	48 ,,	,,
22	Nitrate	0·4	,, ,, 9 ,,	34 ,,	,,
23	,,	0·4	,, ,, 9 ,,	,, ,,	,,
24	,,	0·4	,, ,, 9 ,,	34–45 ,,	,,
25	Acetate	0·4	,, ,, 9 ,,	24 ,,	,,
26	,,	0·4	,, ,, 9 ,,	26 ,,	,,
27	,,	0·4	,, ,, 9 ,,	,, ,,	,,

B 2. Experiment performed on Trout (*Salmo faris*).

1	Nitrate	5	500 c.c. not renewed	48–60 hours	Fatal

B 3. Experiments performed on Goldfish (*Carassus auratus*).

1	Nitrate	250	500 c.c. not renewed	4½– 5 days	Fatal
2	Acetate	10	,, ,,	20 ,,	Not fatal
3	,,	10	500 c.c. renewed alternate days	12 ,,	Fatal
4	Sulphate	25	,, ,, ,,	4– 4½ ,,	,,
5	,,	2·5	500 c.c. not renewed	32 ,,	Not fatal

Table C.

Analysis of the Flood-waters of Rivers Rheidol, Teifi and Meurig—December, 1923.

(Results expressed in parts per 100,000.)

	Rheidol	Teifi	Meurig
Total solid matter ...	4·60	4·40	4·80
Solids non-volatile ...	3·00	2·60	3·00
Loss on ignition ...	1·60	1·80	1·80
Chlorides as Cl ...	1·14	1·14	1·14
Eqt. to sodium chloride	1·89	1·89	1·89
Lead in solution ...	0·04	nil	nil
[pH	6·4	6·2	6·5]

I am indebted to Mr John Evans, F.I.C., Public Analyst for the County of Cardigan, for the figures (exclusive of the last two lines) which appear in the above table, also for the analysis of solid-materials; the tests of lead in solution have been carried out by Mr F. S. Gregory in the Edward Davies Chemical Laboratories, to whose Director, Professor T. Campbell James, my acknowledgments are due for his superintendence of the method employed.

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AN EXPERIMENTAL STUDY OF THE FUNGAL INVASION OF APPLES IN STORAGE WITH PARTICULAR REFERENCE TO INVASION THROUGH THE LENTICELS

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(With Plates I and II and 2 Text-figures.)

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1. Introduction.

It has been shown elsewhere(14) that the fungi which attack apples during storage come into the store as spores on the surface of the apple. In the main, therefore, the species of fungi which will subsequently cause rots are determined by the orchard conditions and by contamina-

tion during transit to store and handling in the packing shed[1]. The degree of subsequent rotting in storage, however, is probably related more closely to the number of fungal spores present than to their species[2]. It is of interest, therefore, to enquire as a preliminary to the study of the factors which control fungal invasion of apples in storage, how densely the fungal spores are distributed on the surface of the fruit before it is placed in storage.

An apple was selected at random from a tray in the packing shed. A piece of string was tied to the stalk of the apple and passed through the opening at the top of a desiccator which had previously been plugged with sterile cotton wool. The desiccator itself had been washed with very hot water and thoroughly rinsed with spirit. The bottom of the desiccator contained sterilised raisin gelatin, still liquid. The apple was lowered into the medium and raised until a film of gelatin had set round it. It was then left suspended in the top of the desiccator.

After two days the apple was found to be covered with a delicate stubble of fungal mycelium growing perpendicular to the surface, and fungal spores, that had been washed off in the dipping, were developing in abundance in the medium. Examination with a lens showed that growth originated fairly uniformly all over the surface, but was distinctly stronger at the lenticels where tufts of mycelium were developed. No attempt was made to isolate all the fungi present. The experiment was concerned only with the quantity and distribution of fungal spores. It showed clearly that an apple when placed in storage may have fungal spores densely scattered over its surface and suggests that they are aggregated more closely in the lenticels.

2. Experimental Technique.

In order to analyse more closely the problem of the distribution of fungal spores upon the apple surface, the following technique was devised. This technique was also used in subsequent experiments.

Small glass tubes with an inner diameter of 3–4 mm. and 2–3 cm.

[1] Under ordinary storage conditions, when the apples are placed in trays in ventilated sheds, a second source of infection arises during storage. Certain species of fungi, *e.g.* Penicillium spp. and *Cladosporium herbarum* fructify and bear spores on the apple after causing a rot, and these spores are dispersed by carelessness in removing rotted fruit, and by draughts and insects. But the increased practice of wrapping the fruit in paper, and the use of cold storage greatly diminishes this source of infection.

[2] The relative importance of the different species of fungi in relation to invasion will be the subject of a subsequent paper. A full account of the actual species that occur has already been given elsewhere by the present authors (9).

long were sealed on to the surface of the apple with luting wax. These tubes were previously sterilised in a dry oven with plugs of cotton wool at either end, one of which was removed when the tube was fixed to the apple. The tubes were then filled with a liquid medium or water, re-plugged and kept under observation. Isolations were made of any fungi developing in the tubes and from any rots developing beneath the tubes. The apples used in the following experiments were Bramley's Seedling and the experiments were conducted at a constant temperature of 15° C. in a humid atmosphere. Control tubes were fixed on to sterile glass slides.

3. Distribution of fungal spores on the surface of the apple and effect of the sterilising agents.

In the first experiment conducted, the object was to estimate the number of the spores present on the cuticular surface of the apple as compared with the number present in the lenticel cavities, to explore the effects of various sterilising agents in killing the spores in either case and to identify the fungi.

For this purpose tubes were fixed on the surface over lenticels and also on areas of the surface apparently free from lenticels. In one set the apples were sterilised in 0·05 per cent. mercuric chloride for three minutes, washed in running tap water and rinsed in spirit; in another set the apples were rinsed in spirit only; and in the last set the apples were unsterilised. Liquid medium was placed in all the tubes.

The result of covering small areas of the surface in this way with liquid medium was that fungal spores present germinated and mycelium was to be seen growing from the bottom of the tube. In the case of the tubes placed over the lenticels the mycelium could often be observed actually emerging from the lenticel crater. After a certain number of days the fungal mycelium was removed, cultured and identified.

Table I gives a summary of the results of this experiment. It shows that the fungi are very numerous all over the surface of the apple, but that those on the surface between the lenticels are more readily killed by methods of disinfection than those occurring actually in the lenticel cavities. For example, the viable spores seated on the surface of the apple between the lenticels were reduced 75 per cent. by rinsing the apple in spirit, while those at the region of the lenticel were reduced only 8 per cent.[1] In the case of apples sterilised with mercuric chloride all

[1] Obviously the tubes over the lenticels cover more free surface than is actually occupied by the lenticel cavity, and, therefore, some reduction in the number of viable spores at the region of the lenticel due to sterilisation is to be expected in the above experiments, even if the spores actually in the lenticels are not affected.

Table I.

Fungi isolated from the surface of apples by placing tubes containing liquid medium over and between the lenticels. Bramley's Seedling from Fen Soil. November, 1922.

	Apples sterilised with spirit		Apples sterilised with mercuric chloride		Apples unsterilised	
	Tubes over lenticels	Between lenticels	Over lenticels	Between lenticels	Over lenticels	Between lenticels
Number of tubes used	50	34	16	15	12	9
Number of tubes showing no growth	1	9	1	15	0	0
Percentage tubes showing growth	98	74	94	0	100	100
Number of fungi isolated ...	65	22	5	0	17	23
(1) *Polyopeus purpureus* ...	28	8	2	—	8	8
(2) Penicillium spp. ...	14	8	—	—	3	6
(3) *Cladosporium herbarum*	6	—	—	—	2	2
(4) *Fusarium fructigenum* ...	6	—	2	—	3	1
(5) *Hyalopus pruinosus* ...	—	1				—
(6) *Dematium pullulans* ...	—	—	—	—	—	2
(7) Stemphylium sp. ...	—	—	—	—	—	1
(8) Unidentified fungi ...	11	5	3	—	1	3
Number of fungi isolated per 100 tubes	130	65	31	0	142	250
Percentage reduction in number of fungi due to method of sterilisation	8	75	78	100	—	—

the fungi on the surface between the lenticels were killed, but in the lenticels themselves a number of spores survived.

It is possible from the results obtained by this experiment to make a rough estimate of the number of spores of rot-forming fungi brought in from the orchard on the surface of the apples used in these experiments. This estimate is, of course, a minimum estimate because one has to assume that all the spores covered by tubes germinated, and that only one spore of each fungus identified in a tube was present in the tube originally.

Taking 12·5 sq. cm. as the area covered by 100 tubes and 150 sq. cm. as the area of the surface of the average apple, and basing our estimate on the figures given in the last column of Table I it would appear that there are in the order of 3000 fungal spores on the surface of a single apple.

It is surprising how large a proportion of the spores present on the surface of the apple appear to lie in the lenticels. For example, in the area covered by 100 tubes the maximum number of fungi developed on

unsterilised apples was 250, when such an area was sterilised with mercuric chloride and spirit no fungi developed if lenticels were absent, but 31 fungi developed when 100 lenticels were present. These figures would indicate that *at least* 31 per cent. of the lenticels contain fungi. Counts of lenticels, visible to the naked eye, indicate that there are approximately 1000 lenticels per apple, which gives us a minimum figure of 300 spores present in the lenticels per apple or 10 per cent. of the total number of spores on the whole surface.

4. Modes of entry to the apple host of fungi under natural conditions.

In order to ascertain how invading fungi obtain access to the apple and to gain some idea of the relative importance of different modes of entry, a careful examination was made of a large number of rots before they had developed very far. It is seen in Table II that there are four modes of entry of major importance, namely, through wounds, the calyx end of the fruit, through the stalk and through the lenticels. Unfortunately the study was made on apples which were held in storage wrapped in oiled papers. It was found subsequently that oiled wraps have the effect of reducing infection through the lenticel. In the table below it will be seen, however, that lenticel invasion is an important mode of entry; had the apples been unwrapped its importance would undoubtedly have been greater.

Table II.

Showing the relative importance of the different modes of entry of fungi to the apple tissue[1]. *Apples wrapped in oiled wraps.* (*Calculated from inspection of* 712 *rots.*)

Mode of entry:			
Through wounds including insect punctures	Through calyx end of fruit	Through stalk	Through lenticel
16 %	36 %	16 %	26 %

In Table III the general result with regard to infection through the lenticels shown in Table II above is differentiated according to the origin of the apples which were obtained from different localities and soils and from trees of different ages. It appears from this table that lenticel invasion is more important in apples from some localities than from

[1] The less important modes of entry in apples wrapped in oiled papers include fungi following scab, capsid infection, internal breakdown, sieve bruises and to a very small extent—in the case of apples in oiled papers—apple scald.

others and more important in the case of fruit off young trees than in the case of fruit off old trees.

Table III.

Percentages invasion through lenticels in apples from different localities. (Calculated from inspection of 264, 129, 100 *and* 219 *rots respectively.)*

From locality 1 (fen land)	38
2 (silt land, young trees)	30
(silt land, old trees) ...	13
3 (chalk land)	17

Table IV gives a more detailed analysis of the results tabulated in Table III, and distinguishes between different parasitic fungi. It suggests that some fungi attack through the lenticel more readily than others, and that species of Polyopeus and Pleospora are outstanding in this respect.

Table IV.

Percentage invasion through lenticels in the case of different fungi[1].

Apples from locality:	Fusarium	Penicillium	Polyopeus	Pleospora
1 (fen)	16	55	65	55
2 (silt, young trees)	10	23	91	100
(silt, old trees) ...	25	10	75	0
3 (chalk)	0	10	33	16

The large number of infections through the calyx shown in Table II is not surprising when we consider its character and position. Being often a sunken area it is likely to collect dust and spores in the orchard. It is also likely to collect and hold moisture in the store, especially when the apple rests on this end, and the moisture would promote spore germination and growth. Further, some apples have an open communication from the calyx to the core, and for this reason are more susceptible to invasion at this area.

Wound rots and stalk rots are analogous because observation has shown that rots developing at the stalk generally occur when this has been broken off at the time of picking.

5. Experimental analysis of the factors affecting lenticel invasion.

The study of this method of invasion is of practical importance on account of the high proportion of rots that originate at the lenticel. It is

[1] These figures are calculated on the basis of the total number of invasions by each specific fungus in the case of the apples from each soil.

also the most interesting method biologically because invasion would appear to be dependent mainly upon the physiological condition of the parasite and of the host. As has been shown above, the surface of the apple carries numerous spores of parasitic fungi in the cavities of the lenticel and the lenticel in the apple is usually a simple opening in the cuticle (see Appendix).

In approaching experimentally the problem of lenticel invasion we have set out from the formal concept that fungal attack depends on (1) germination and growth of the fungus outside the host, (2) mechanical resistance of the host to invasion, (3) physiological resistance of the host to invasion.

(a) *Nutrient.*

The following experiment was designed to answer the question how far the spores present in the lenticels invade the apple given favourable conditions for initial germination and growth. In this experiment the tube technique described above was employed, liquid medium being placed in the tubes attached to the apple. The fungi developing in the medium were isolated at the end of a week and subsequently identified. Rots began to develop about the same time under the tubes. The tubes were removed, but the rots were kept under observation for two or three weeks longer before isolations were made. The results are summarised in Table V. They show clearly in the first place that, given the conditions for active germination and growth, the fungi present in the lenticels are

Table V (Dec. 1922).

	Total number of fungi growing in media per 100 tubes	Percentage tubes showing growth	Percentage Invasion, i.e. number of apple rots per 100 tubes	Invasion Ratio, i.e. ratio of number of fungi present to number of rots	Number of fungi isolated from rots but not corresponding to those isolated from covering tubes, per 100 tubes
Over lenticels ...	141	100	75	·41	42
Between lenticels	211	100	33	·16	0

capable of invading the apple. Secondly, while in every case fungal growth appeared in the medium over lenticels in only 75 cases out of 100 did rots subsequently develop on the apple. Thirdly, a certain number of rots developed when tubes were placed, as far as could be determined by eye judgment, *between* the lenticels; but the number of rots that appeared in this case is much smaller than when the tubes were placed over the lenticels. Finally, the results given in the last column would seem to indicate that when a fungus is isolated from the medium in the tube above the lenticel, it is not necessarily the only one present.

(b) *Mechanical and physiological resistance.*

The results enumerated above give rise to the following questions. Does invasion actually take place through the cuticle or are some of the tubes between the lenticels really covering gaps of microscopic size? Secondly, why is the invasion through the lenticel only of the order of 75 per cent.? Is this due to physiological immunity or to purely mechanical resistance? As is shown in the appendix, the lenticels are often blocked with débris, suberised or corked off, and this cannot be determined by inspection.

Taking these queries in order, the first was investigated further by the tube method. It has already been shown that sterilising agents reduce the number of fungal spores present to a greater extent when they are situated on the free surface of the cuticle than when they occur in cavities or gaps in the cuticle. Now, if the percentage invasion (33 per cent.) and the invasion ratio (0·16) obtained with unsterilised apples between the lenticels (Table V) is in the main due in reality to the fact that small lenticels or gaps in the cuticle have escaped detection, then we should expect the following result when the experiment is repeated with sterilised apples. The total number of fungi developing in the superimposed media between the lenticels should be greatly diminished, but the total number of rots developing (percentage invasion) under tubes should not be reduced to the same degree. Further, as a result of these two effects, the invasion ratio, *i.e.* the rots per 100 fungi present, would be increased. Table VI, which gives the results of an experiment with sterilised apples, shows that these expectations are realised and we can consequently state with a fair amount of certainty that the rotting induced in unsterilised apples by placing tubes between the lenticels is largely due to the fact that gaps in the cuticle have escaped observation in setting up the experiment. We cannot, however, critically negative the possibility of actual penetration of the cuticle by the fungus[1].

To proceed next to the second question, namely, why does invasion occur in some cases and not in others when the fungi are growing vigorously in media superimposed on the lenticels. It has been shown that a certain number of lenticels are mechanically blocked and that these cannot be distinguished by external inspection. It might be supposed that the 25 per cent. of failure to invade was entirely due to

[1] As will be seen in the appendix on the structure and development of the lenticel, examination with a lens often reveals the presence of lenticels or gaps in the cuticle invisible to the naked eye.

Table VI (Dec. 1922).

	Total number of fungi developing in media, per 100 tubes	Percentage Invasion, i.e. number of apple rots per 100 tubes	Invasion Ratio, i.e. ratio of number of fungi present to number of rots	Number of fungi isolated from rots but not corresponding to those isolated from covering tubes, per 100 tubes
Apples sterilised with spirit:				
Tubes over lenticels ...	127	77	0·49	31
Tubes between lenticels	86	25	0·25	14
Apples sterilised with mercuric chloride:				
Tubes over lenticels ...	62	31	0·38	19
Tubes between lenticels	0	0	0	0

mechanical resistance and that physiological resistance or immunity of the underlying tissue played no part.

We can submit this point to experimental verification by carrying out an invasion experiment with tubes containing medium over lenticels definitely known not to be blocked. Apples were exposed for a few hours to an atmosphere containing dilute ammonia vapour. As a result of this treatment the open lenticels of the apple show up as obvious brown spots, and many previously invisible to the naked eye or the low power lens appear.

Only marked lenticels were selected after this treatment of the apple and covered with tubes containing liquid medium and in addition the tubes were inoculated with spores of various species of apple rot fungi. The conditions of this experiment may therefore be regarded as eliminating every possible bar to invasion saving only physiological resistance of the living tissue of the apple. The result obtained was as follows:

February, 1923.

Control ... 34 per cent. failure to invade with unselected, untreated lenticels.

Experiment ... 15 per cent. failure to invade with selected lenticels, known to be open.

This result does not indicate that failure to invade at the lenticels can be attributed solely to mechanical blocking. It appears that physiological resistance may be operative to a certain extent.

(c) *Moisture.*

We have now seen that, given the conditions for active germination and growth, the spores of apple rot fungi present on the surface of the

apple penetrate immediately through the lenticel and invade the apple with great ease. The important factors controlling fungal penetration through the lenticel in storage would therefore appear to be those which affect the germination and growth of the fungal mycelium outside the host, namely, moisture, nutrition and temperature.

Table VII (April, 1923).

	Water				Media				Control			
	Over lenticels		Between lenticels		Over lenticels		Between lenticels		Over lenticels		Between lenticels	
	Percentage tubes showing growth	Percentage invasion	Percentage tubes showing growth	Percentage invasion	Percentage tubes showing growth	Percentage invasion	Percentage tubes showing growth	Percentage invasion	Percentage tubes showing growth	Percentage invasion	Percentage tubes showing growth	Percentage invasion
After 2 weeks	42		0		79		63		0		0	
„ 3 „		7		0		17		0		0		0
„ 5 „		21		8		33		7		0		0
„ 7 „		26		10		48		10		0		0

The effect of moisture in promoting fungal growth and initial invasion cannot be considered apart from the effect of nutrient, because exosmosis may be expected to occur through the thin walled cells surrounding the lenticel cavity into any external moisture that may be present. An attempt was made to investigate by means of Brown's conductivity method(7) the amount of such exosmosis. Figs. 1 and 2 give typical curves obtained by plotting the conductivity of the superimposed liquid against time, when pure water was placed over lenticels. These indicate that exosmosis occurs and that both in the case of lenticels injured by ammonia and in the case of normal lenticels, nutrient accumulates in the external water until a certain low equilibrium concentration is reached and that this equilibrium concentration is approximately the same whether the lenticels have been injured or not[1]. If, after this exosmosis equilibrium has been reached, pure water is applied more exosmosis takes place and approximately the same equilibrium concentration is again reached (Fig. 2).

From these facts we may conclude tentatively that the concentration of nutrient occurring in any film of moisture in the cavity of the lenticel is more or less independent of the amount of external water or moisture present within broad limits: and we can therefore further conclude that, if invasion and rotting is found to be increased by the addition of water, lack of available moisture for the fungus has previously been a limiting

[1] From the conductivity experiments an interesting fact came out, namely that exosmosis equilibrium from different lenticels varied considerably.

factor. The following experiment, the results of which are shown in Table VII, was carried out to study directly by the tube method the effect of water over lenticels[1]. This experiment shows that when drops of water were superimposed over lenticels the percentage invasion was increased from 0 per cent. in the untreated controls to 26 per cent. when water was added. When full strength medium was used the percentage invasion was further increased to 48 per cent., almost double that occurring with water alone. In view of the consideration outlined above

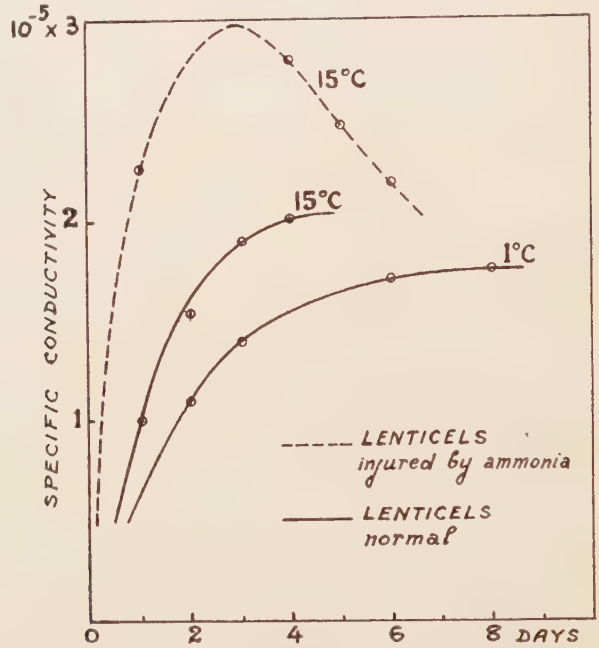

Fig. 1. Showing rise in concentration of exosmose to a low equilibrium concentration, the external liquid being unchanged. (Specific conductivity of water used was $\times 10^{-6}$.)

we must conclude that failure to invade under natural conditions in storage is due in part to lack of available moisture for the fungus outside the host and in part to the unsuitable quality and insufficient concentration of the exosmose nutrient.

It may be observed before passing, that the results shown in Table VII constitute a clear demonstration of the fact that exosmosis of nutrient occurs through the lenticel, but not through the cuticle. In tubes containing water placed over lenticels fungal growth appeared in 42 per

[1] The apples used in this experiment were rinsed in spirit.

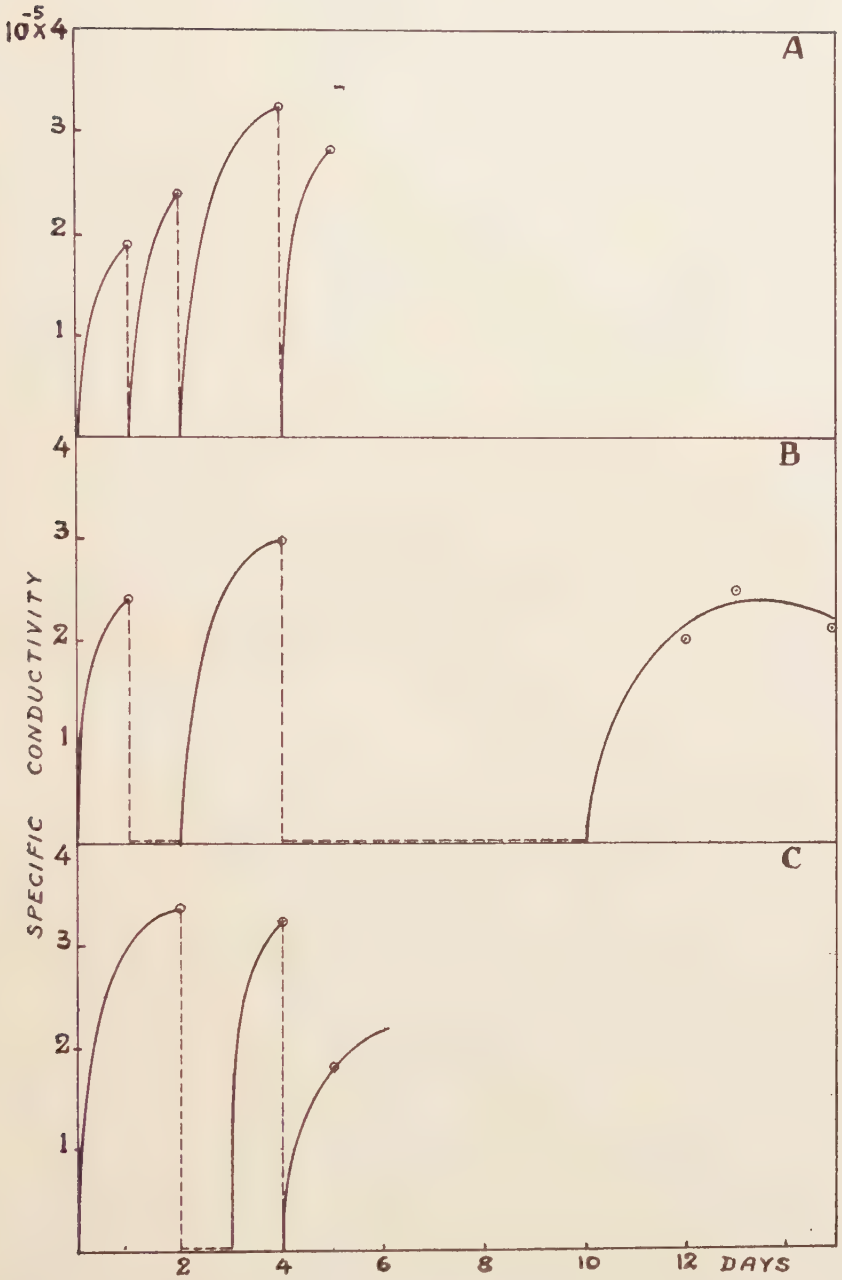

Fig. 2. Showing that the renewal of the external liquid between each reading does not greatly affect the exosmosis equilibrium concentration reached, and thus indicating that the amount of external moisture does not affect to any great extent the concentration of nutrient available for the fungus outside the living cells. (Specific conductivity of water used $\times 10^{-6}$.) *A*, *B* and *C* three typical experiments.

cent. of them. When on the other hand the tubes were placed between the lenticels fungal growth did not appear in a single tube. That fungal spores were present in both cases is shown by the fact that, when tubes containing media on similar apples at the same time were used, 79 per cent. and 63 per cent. growth was obtained on and between the lenticels respectively.

(d) *The storage age of the apple.*

In storage at constant temperature and moisture the percentage invasion per unit time increases as the storage age of the apple advances. The experimental evidence dealt with above has indicated that, when mechanical and physiological resistance are eliminated; the growth of the fungus outside the host and consequent invasion are controlled by moisture and nutrient. As there is no evidence that the amount of moisture varies with the storage age, we must conclude that the increase in the invasion percentage which occurs as the apple grows older is mainly due to an increase in the amount or suitability of the nutrient escaping by exosmosis. Investigation is in progress to determine what changes occur, with the age of the apple, in the quality of the exosmose and in the exosmosis equilibrium concentration in external moisture.

We may consider next what effect the increasing age of the apple may have upon the factors of mechanical and physiological resistance. The various experiments described above afford some indication that one or both of these factors become more potent in preventing invasion as the age of the apple advances. For example, as will be seen from Table VIII, 75 per cent. invasion was obtained when liquid medium was placed over the lenticels in December, 1922, as compared with only 66 per cent. in February, 1923; while in the following April, 1923, the falling off in the percentage invasion, when full medium was given, was still more pronounced[1].

Table VIII.

Percentage invasion on the application of media in tubes over lenticels.

	December	February	April
Unsterilised apples or apples sterilised with spirit followed by heavy inoculations	75	66	—
Apples sterilised with spirit	77	—	48

[1] Any possible falling off with time in the viability of the spores on the apple has not yet been investigated.

6. Lenticel spotting of apples in storage.

(a) *Historical.*

Spotting of apples in storage, the spots developing at the lenticels, has been described by several investigators in this country and in America, and this phenomenon must now be considered in relation to lenticel invasion.

In 1913, Scott and Roberts (11), working in America, wrote as follows:

> A lenticel usually forms the centre of each spot. The spots though seldom more than skin deep detract greatly from the appearance of the apple and afford a place of entrance for decay fungi. They are dark brown in colour, more or less circular in outline, at first scarcely depressed, later becoming considerably sunken and vary from one-eighth to one-fourth of an inch in diameter....Nearly 400 cultures of the diseased spots have been made in various ways and on various media, but no organism has been isolated with any degree of consistency. A species of Alternaria often occurred in cultures from fruit grown in the eastern part of the country, but the north-western grown fruit were almost barren.

These authors concluded that they were dealing with a functional or physiological disease of the apple and they adopted the name of Jonathan Fruit Spot.

In some seasons a similar spotting at the lenticel is a very prevalent disease in storage with many varieties of English apples. Barker (1) investigated an outbreak which occurred in 1914 and the following is a summary of his observations.

There was no sign of the trouble at the time of gathering the fruit. The spots first appeared on the surface of the fruit as small brownish specks, which gradually enlarged. In many cases the spots remained quite small and not larger than a pin's head. Towards the centre of each spot the skin of the apple showed a break and examination showed that these breaks corresponded to lenticels. Fungal hyphae were present in all the cases examined. Three or four species were isolated. Barker concluded that in certain seasons apples are peculiarly liable to attack through the lenticels by the hyphae of certain fungi capable of causing a local rot.

Spinks (12) in 1916, continuing Barker's study, concluded that the fungus enters the apple through the lenticel some time before the first spot appears. The fungus for a time makes very little growth and only produces a noticeable spot when the apple has reached a suitable age of ripeness. The spot then grows rapidly.

Brooks and Cooley (3) in 1919 gave a further account of the spotting

disease of apples which Scott and Roberts had termed Jonathan Fruit Spot. Their observations show that:

> The spots usually occur at the lenticels. In the early stages of the disease only the superficial colour bearing cells are involved, and the spots are rarely more than 2 mm. in diameter. Later they may enlarge to 3–5 mm. becoming slightly sunken and spread down into the tissues of the apple to a considerable depth. In the later stages of this disease rot fungi are often present.

These authors maintain that fungi are not present in the initial stage of the disease which they consider to be physiological in origin. They suggest that a close relationship exists between the fundamental cause of Jonathan Spot and of Apple Scald.

In 1921, Horne(8), writing on the spotting of apples in Great Britain, states:

> A careful examination will show a complete series of stages from discoloured lenticels to spots. The production of new spots goes on continuously throughout the storage season. Over 200 spots were counted on one specimen. In some cases spots appear early in the season and remain without development. In others there is a steady increase in size. In over 20 varieties out of 90, rotting that could be traced to spotting had set in before the varieties were out of season.

From the above summary of the more important literature on lenticel spotting it would appear that all investigators agree that spots in their advanced stage frequently become rapidly developing fungal rots, but that a long initial phase occurs during which spots only increase in size very slowly, if at all. It may be said that the consensus of opinion in America appears to favour the view that the disease during its initial phase is the result of some physiological disturbance at the region of the lenticels and not directly due to fungal attack. In Great Britain, however, up to the present, investigators have been inclined to conclude that lenticel spotting is a direct result of fungal infection and that the slow development is due to the physiological resistance of the apple tissue[1].

(b) *Is lenticel spotting a functional or a fungal disease in origin?*

The experiments carried out by the present authors with English apples support the view that lenticel-spotting is a functional disease in origin.

[1] There appears to be a marked similarity between the lenticel spotting of apples and pit rot of potatoes as described by Pethybridge (10). Affected tubers show dark circular, depressed areas on the surface, and in the centre of each depression an "eye" occurs or more commonly a lenticel. The disease occurs only in the storage pits, and it is not caused by fungi or bacteria although these may be of secondary occurrence.

In the first place the observations of Scott and Roberts, and of Brooks and Cooley, have been confirmed, to the effect that fungi cannot always be isolated from spots during the initial phase of the disease. Table IX gives the result of a number of isolations that were made from typical lenticel spots, from 1–2 mm. in diameter (see Pl. I, fig. 2).

Table IX.

Showing the result of isolations from lenticel spots during the season 1920–21.

Total number of spots examined	Number of spots yielding one or more fungi	Number of spots yielding no fungi
157	117	40

The following were the fungi isolated:

Cladosporium herbarum ...	43
Unidentified spp.	33
Polyopeus spp.	21
Pleospora pomorum	14
Hyalopus pruinosus	5
Penicillium spp.	4
Fusarium spp.	2
Alternaria tenuis	1
Diaporthe perniciosa	1

Horne, also working with English apples, has reported a large number of failures to isolate fungi from spots, but states that: "In some cases this was due to the use of liquid apple extract and in others to over sterilising in mercuric chloride." Particular care was taken by the authors to avoid over sterilising and various ordinary agar media were used.

More conclusive evidence than the above, however, to the effect that the origin of lenticel spotting is functional, is afforded by an experiment in which it was found that methods, which control the appearance of the functional disease known as apple scald (3, 4, 5, 6), also control the development of spotting. The investigations of Brooks, Cooley and Fisher have established the empirical fact that scald is controlled by wrapping the fruit in papers impregnated with refined mineral oil. The present writers have found the same measures are effective in preventing the development of lenticel-spotting. (See Table X.)

Table X.

The control of lenticel spotting of Newton Wonder and of Bramley's Seedling by wrapping the fruit in paper impregnated with refined mineral oil.

	Percentage of apples spotted		
	When unwrapped	When wrapped in ordinary tissue paper	When wrapped in oiled paper
1921.			
Newton Wonder, from locality 1 ...	71	64	0
,, ,, ,, ,, 2 ...	33	16	0
Bramley's Seedling (from fen soil) ...	—	57	0
1922.			
Bramley's Seedling (from chalk soil)	53	—	15

(c) *The transition from the initial stage of spotting to that of active fungal invasion.*

In the initial stage of spotting although active invasion is delayed, the germination of spores may take place and mycelium may be present. Both Barker and Horne describe the appearance of the fungi in microscopic sections through small spots. Mycelium is stated to be present in the air spaces, the hyphae penetrating between the cells and enveloping them in a web of filaments.

It has already been shown that provided sufficient moisture and nutrient is present invasion follows immediately and we have therefore formulated the following propositions[1] as giving a comprehensive schema of the relations that determine the occurrence of active invasion.

1. That the critical stage of the fungus conditioning invasion is not necessarily the germination of the spore but the attainment of a certain vigour of development.

2. That the *local* physiological breakdown of the apple tissue in the neighbourhood of the lenticels may supply sufficient moisture and nutrient for the fungus to germinate, but not enough to enable it to reach the critical vigour of development required for active invasion.

3. That, when local physiological breakdown at the lenticels occurs, a competition takes place between the underlying healthy apple tissue and the fungus for the available moisture and nutrient.

[1] *Wound inoculations and the tube technique of inoculation.* It is often necessary to establish the parasitism of a species or strain of fungus by inoculation. From a general application of the propositions stated above, which have arisen from the evidence given in the foregoing paper, it follows that the failure of a potential parasite when inoculated by a wound should often occur. Even an internal wound rapidly dries up, the adjacent healthy cells adsorbing the moisture liberated by the dead cells, as is well seen in the case of Bitter pit and Brown heart. Inoculation by the tube technique has therefore been the method adopted by the present writers in their work on apple rot fungi (9).

4. That the fundamental controlling factor is the exosmosis equilibrium concentration of the general mass of apple tissue.

Summary.

The spores of rot forming fungi on the surface of the apple and in the lenticel cavities have been studied as regards their distribution and number.

The natural modes of entry of apple rot fungi have been described and the relative frequency of their occurrence assessed.

The factors controlling lenticel invasion in storage have been analysed by a new experimental technique.

The literature dealing with lenticel spotting of apples has been reviewed; the cause of spotting has been distinguished as functional rather than fungal and the general name "Lenticel Spot" has been proposed for the disease.

APPENDIX

Structure and development of the Lenticel.

In the mature apple the lenticels are small irregular gaps in the cuticle. The torn edge of the cuticle is often bent up and forms the margin of a small crater (Pl. II, fig. 2). The size of the lenticels varies to a considerable extent; the majority have an opening of about 60μ, but others show a diameter of 100–300μ. The cells within the crater are not modified in any way, unless as sometimes happens a layer of cork cells is formed. The subepidermal cells have thick, slightly cutinised cell walls and the lenticel cavity is usually confined to this region.

In the young apple there are no lenticels; connection with the atmosphere is provided by the stomata (Pl. II, fig. 1), which are remarkably few in number. In sections of a small cube of tissue of Bramley's Seedling cut in June, 1913, no interruptions of any kind were seen in the epidermis except for the hairs. At this stage the cuticle is quite thin (2–4μ) and not suberised. In July, while the apples are still green, the lenticels appear as white spots which are very conspicuous. The most rapid growth of the apple takes place in June and July; at the end of this period the apple is mature in its general characters, though not in size. Lenticels are numerous, the hairs have disappeared except very close to the stalk and calyx, and stomata are very rare indeed. According to Brooks mature apples still possess stomata, but we have seen one in one case only (Bramley's Seedling, August, 1923). Zschokke (13) states that they are rare. The idea first brought forward by Zschokke

that the formation of the lenticels is due to the simple tearing of the cuticle is supported by a microscopic examination of these structures viewed from above. The outline of this cavity is very irregular, often stellate in appearance. Such tearing of the cuticle would be expected were the enlargement of the apple greater than the rate of growth of the epidermis. The stomata and the pockets of the hairs would naturally yield first to the strain, so that these would tend to disappear and be replaced by the lenticels. Further observations show that there is a greater increase in tangential dimensions of the epidermal cells during the growth of the apple than in the radial dimensions, in the case of Bramley's Seedlings, 8μ to 25μ and 15μ to 18μ respectively.

At the same time it is certain that a considerable amount of growth of the epidermis takes place which compensates partially for the increase in diameter. As shown by Zschokke the epidermal cells divide and increase in number. Also, round many of the stomata and hair-gaps cell-division takes place along lines perpendicular to the directions in which tearing would take place. Thus the size of the lenticel crater is prevented from becoming too large.

All the stomata and hair-gaps do not subsequently function as lenticels, for cork formation is of frequent occurrence (Pl. II, fig. 3). To the eye they would appear to be functional lenticels owing to the torn-up edges of the cuticle persisting. The amount of cork formation varies with the time of the year and with the variety, but the character is possessed by all varieties and no constant difference has been observed between them. Wound cork is rarely formed.

The density of distribution of the lenticels increases from the stalk end to the calyx end. From counts made of areas of 1 sq. cm. in different parts of the apple, the numbers of lenticels present on the stalk end, middle region and calyx end respectively are 1, 2, 4.

An average of counts made of lenticels in 1 sq. cm. surface (*a*) at calyx end, (*b*) at stalk end on 20 apples (Bramley's Seedling off fen soil), were as follows:

Lenticels per sq. cm. of surface.

At calyx end of apple		At stalk end	
Visible to naked eye	Visible under lens	Visible to naked eye	Visible under lens
12	19	4	11

An estimate of the efficiency of the lenticel is not possible by external examination only, since the nature of the cavity, whether it is closed

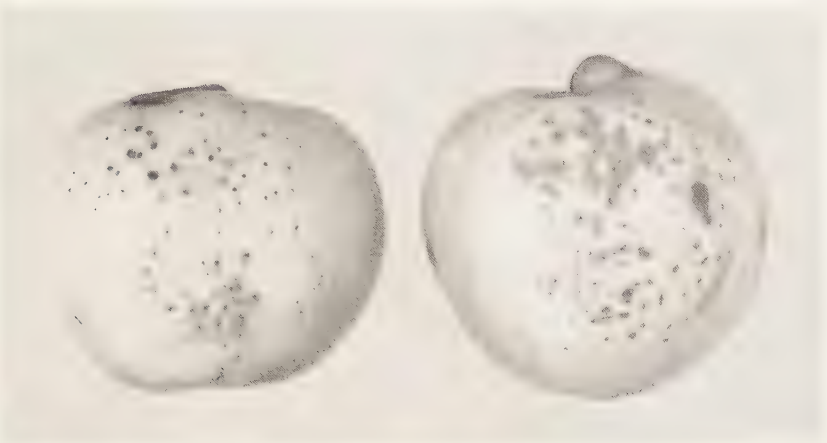

Fig. 1.

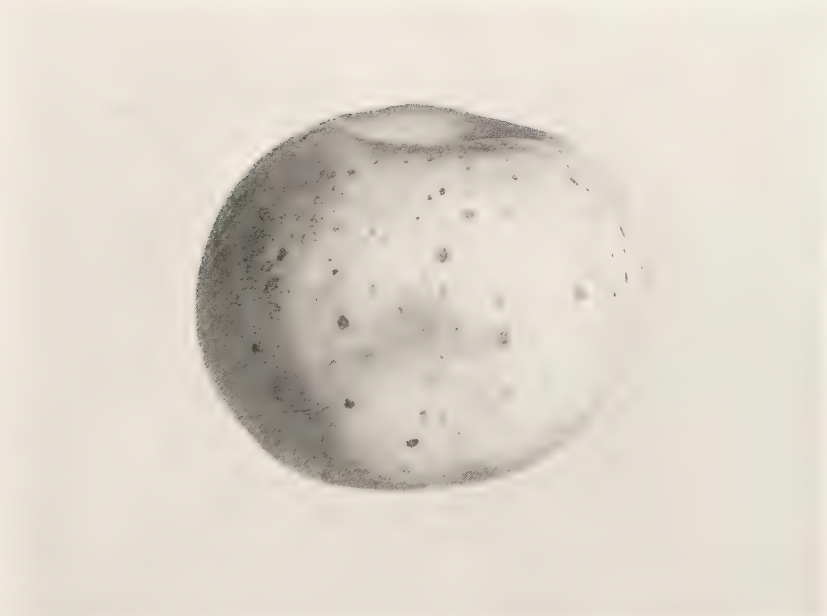

Fig. 2.

KIDD & BEAUMONT.—Fungal Invasion of Apples in Storage. (pp. 14–33.)

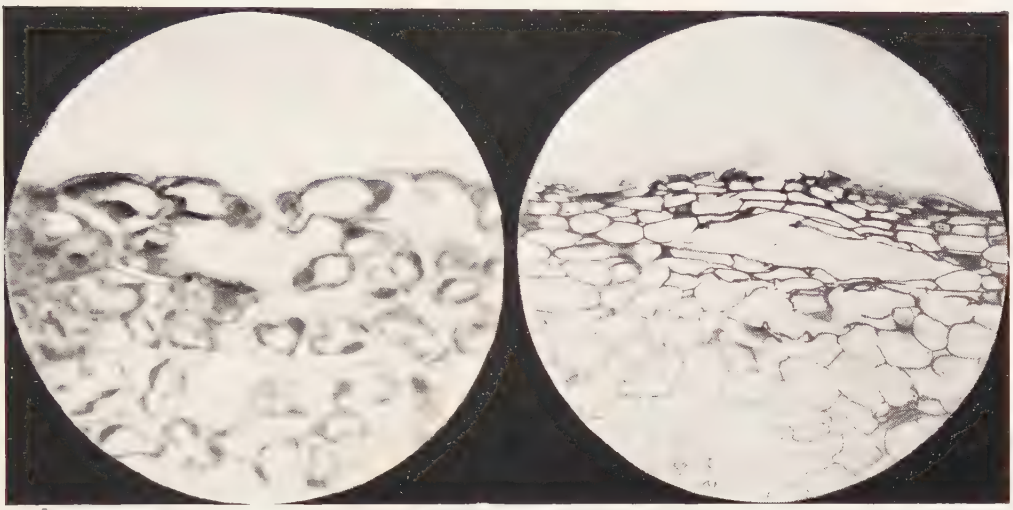

Fig. 1. Fig. 2.

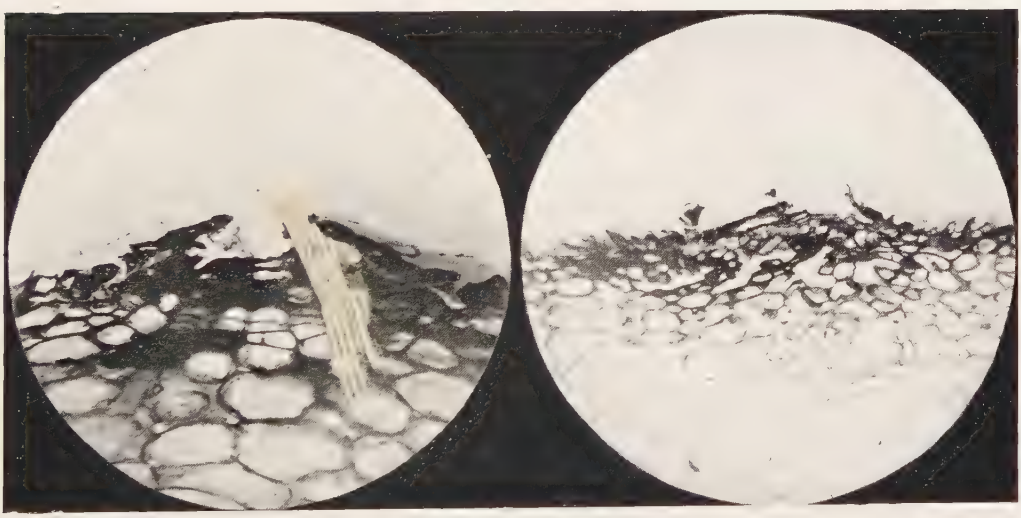

Fig. 3. Fig. 4.

KIDD & BEAUMONT.—Fungal Invasion of Apples in Storage. (pp. 14–33.)

with cork or not, or how large it is, cannot be seen by this means. If apples are immersed in water under reduced pressure the more open or more porous lenticels emit a rapid stream of large bubbles. The less open give rise to smaller bubbles coming off more slowly, or only after the pressure has been further reduced. There is no correlation between the size of the lenticels and their porosity.

The open lenticels show up better when the apple has been partially evacuated under water containing methylene blue. When this is done it is frequently seen that many of the prominent brown lenticels are not stained at all.

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EXPLANATION OF PLATES I AND II

Plate I.

Fig. 1. Lenticel spotting on Newton Wonder Apples (controlled by oiled wraps).
Fig. 2. Lenticel spotting on Bramley's Seedling Apples (controlled by oiled wraps).

Plate II.

Fig. 1. A stoma on young apple.
Fig. 2. A normal lenticel.
Fig. 3. A lenticel with cork formation.
Fig. 4. An early stage of a lenticel spot.

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INFECTION EXPERIMENTS WITH WART DISEASE OF POTATOES. *SYNCHYTRIUM ENDOBIOTICUM* (SCHILB.) PERC.

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(With 1 Text-figure.)

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INTRODUCTION.

Investigations directed towards the control of wart disease have hitherto been carried out along two main lines. On the one hand the ultimate aim has been to destroy the parasitic organism *Synchytrium endobioticum* in the soil and so eliminate the disease, and, on the other hand, to render it innocuous by cultivating varieties of potato immune to the disease. It has been clearly shown that the winter sporangia of the parasite survive in the soil for many years in the absence of the potato plant, so that the disease cannot be eradicated from the soil by

any system of crop rotation. Soil sterilisation is the one method of destroying the parasite that seems to offer any hope of success. Although such treatment has often been attempted and has given mostly negative results, in a few cases successful soil sterilisation has been achieved. This has not yet proved feasible on a large scale, but its possibilities are now being extensively studied. At present the only practicable method of controlling wart disease is to grow immune varieties of potato. Plant breeders are therefore actively engaged in trying to produce new types which shall combine the good qualities of some of the best susceptibles, such as early maturity, high yield, good flavour and cooking quality, with immunity. Every year some hundreds of new varieties raised from seed are tested for immunity to wart disease at potato testing stations, such as that at Ormskirk, in Lancashire.

The varieties which prove susceptible are discarded, while the immunes are further tested and the best of them selected to be put on the market.

The study of wart disease of potatoes, whether it is directed towards soil sterilisation or towards the production of new immune varieties, and the latter includes the fundamental study of the genetics of immunity to wart disease, has hitherto been very seriously hampered by the lack of effective experimental methods. In work on soil sterilisation the rational sequence of study whereby conditions, chemical and physical, which bring about the death of the sporangium are determined first *in vitro*, then on a pot scale, and lastly in the field, has not been possible. The difficulty experienced in inducing germination of even a small percentage of sporangia under observation and so of testing their viability rendered *in vitro* tests impracticable.

Pot experiments, though they have been used to a limited extent in soil sterilisation work have proved so unreliable that they have been largely discontinued. In some instances good results involving infection by wart disease of every control plant, have been obtained in pots during spring and summer, the normal season for infection, but in other cases at the same time of the year under apparently similar or equally favourable conditions, little or no infection has been found. For instance, potatoes in pot experiments put up by Gimingham and Spinks (3) in the summer of 1919 were all well infected. At Rothamsted in two successive summers, pot experiments carried on out-of-doors showed in the first season infection in every control plant and in the second, complete absence of infection. In 1920 a set of experiments in a glasshouse at the Potato Testing Station at Ormskirk failed completely because very little

infection appeared even in the controls. Again, in 1921, two sets of experiments involving altogether about 200 pots, each holding 60 lb. of soil, put up by Dr W. B. Brierley, Mr W. A. Roach and myself in a glasshouse, had respectively only three out of seven and six out of ten control plants infected and these for the most part not heavily. Similar experiments carried out in the open in 1922 failed completely through lack of infection and yet the soil used in all these cases came from a plot of land at Ormskirk regarded as one of the most heavily infected areas in the world. Hitherto, therefore, pot experiments have been of little use in research on soil sterilisation and thus a relatively simple and economical method of selecting the best chemicals for testing out on a field scale has not been available.

The only method which is regarded as reliable for testing the immunity or susceptibility of new varieties of potato involves growing them in the field. At Ormskirk the standard method is to grow forty tubers of each variety in field plots on land heavily infected with wart disease. Those which show no infection the first year are cultivated again in the following season and if again free from wart disease are officially listed as immune. If as in the hot dry summer of 1921 and to a lesser extent in 1923, seasonal conditions are unfavourable to the development of the disease infection may be so slight that the tests must be repeated in full or in part the following year. In a year such as 1924, however, when the climatic conditions were exceptionally favourable for the development of wart disease the average infection of Arran Chief, one of the most susceptible varieties of potato in certain heavily infected trial plots at Ormskirk, was about 80 per cent.

The method of field testing for immunity has up to the present proved a reliable one, but its great disadvantage lies in the length of time and expense involved. At least one year is required to raise the necessary tubers from seed after which susceptibility may be shown the following season, but immunity cannot be proved in less than two and in the case of an unfavourable season, three years. A more rapid method of testing the immunity or susceptibility of varieties and tubers is therefore needed.

In the absence of any anatomical criterion or known biochemical reaction which might serve as an index to immunity, the direct method of actual infection with *Synchytrium* is the only means for testing this quality. The standardisation of some method for effecting this more rapidly than is done in the field and by a method less subject to seasonal variation is thus required.

The present communication embodies the results of a somewhat empirical study of some of the conditions and factors controlling infection by wart disease of potatoes. The work is related to and to a large extent has directly grown out of the practical needs and difficulties in a larger study of soil sterilisation with regard to wart disease in progress at Rothamsted. The possibility has been kept in view that by discovering the conditions that favour infection a reliable method for infecting susceptible tubers might be obtained, in the first place, to provide a method of pot experimentation as a laboratory basis for soil sterilisation work and, secondly, to provide a quick method of testing immunity or susceptibility to wart disease.

The communication is divided into two parts, the first of which treats of infection of the host brought about by winter sporangia in the soil, and the second of infection by means of summer sporangia in the laboratory. The work is incomplete but the possibility that the results might be of immediate use in other directions, particularly in the testing of immunity or susceptibility, appeared to justify publication at the present stage.

INFECTION BY WINTER SPORANGIA IN THE SOIL.

TECHNIQUE.

Throughout these experiments, except where otherwise definitely stated, the following procedure was adopted:

Tubers of Arran Chief, one of the most susceptible varieties of potato known, were grown singly in ordinary flower pots with a top diameter of 7 inches, each holding about 3 lb. of soil. These pots were found to be large enough to allow of moderate growth, while they were small enough to be conveniently used in large numbers[1]. Each pot stood in a saucer of 7 inches diameter. The soil used consisted of a mixture of heavy field soil from Rothamsted, with sand, and an organic material consisting of broken chaff and straw and known as "cavings," all passed through a sieve with a $\frac{1}{4}$-inch mesh and mixed in the following proportions:

Soil	...	...	37·5 per cent.
Sand	...	...	50 ,,
Cavings	...	...	12·5 ,,

[1] At Ormskirk in 1921 and 1922 large glazed pots 12 × 12 inches each holding 60 lb. of soil were used by Dr W. B. Brierley, Mr W. A. Roach and myself. Growth though possibly better than in the 7-inch pots was not sufficiently so to make the use of the former worth while in view of the far greater cheapness and convenience of the small pots and their satisfactory experimental results.

The sporangial material which was collected in the late summer and early autumn consisted of warts together with a certain amount of attached decomposed tuber. This material was air-dried, broken up in a mincing machine, passed through a sieve of 3 mm. mesh and mixed well together. A definite quantity, measured by volume, of sporangial material was added to each of 15 lb. or 30 lb. of the soil mixture described above, and well incorporated.

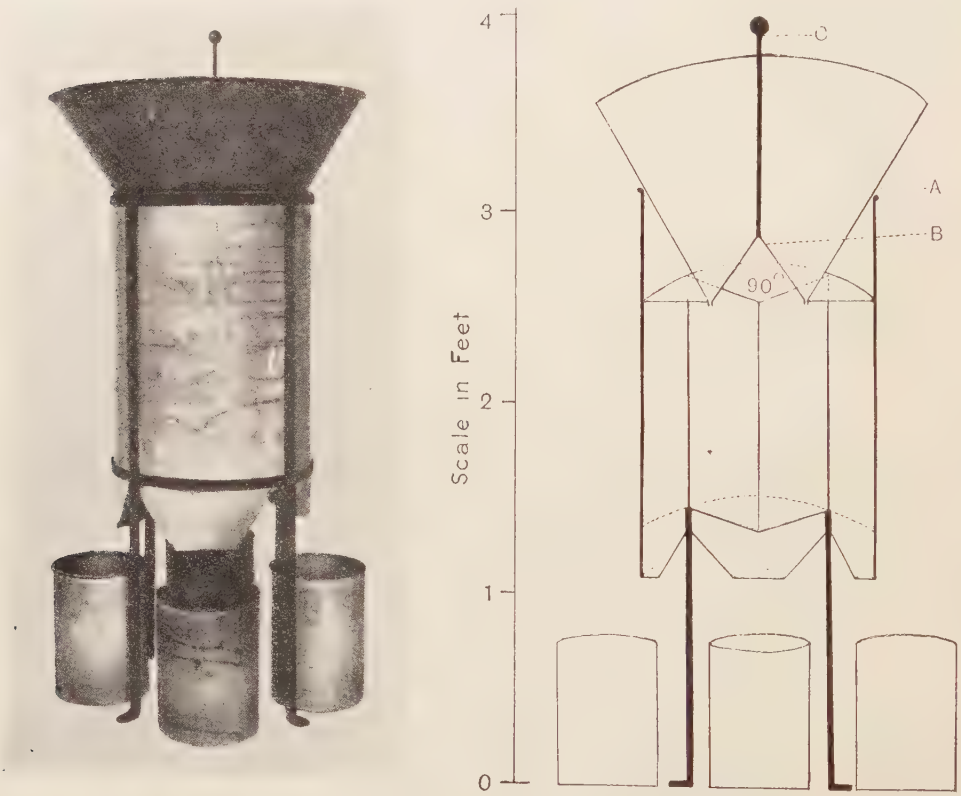

Fig. 1. Mixing apparatus with diagram of longitudinal section.

In the earlier experiments the sporangia were mixed with the soil by hand, but later a less laborious and more efficient method was adopted by use of the soil sampler shown in Text-fig. 1[1]. It is constructed of galvanised iron and consists of a main cylinder divided by means of two

[1] I am indebted to Dr B. A. Keen for drawing my attention to this type of apparatus, which has previously been used in certain sampling operations, and for suggestions as to the manner of construction.

diametrical plates into four quarters each of which leads into a basal spout. A funnel *A*, placed on top of the main cylinder, has its lower end blocked by means of a conical plug *B* attached to a rod *C*. The soil and sporangia are put into *A* which will hold about 30 lb. of soil. *B* is then drawn upwards by means of *C* and the soil and sporangia fall and are divided by the plates into four portions which issue from the spouts into receptacles. The four portions of the soil are then returned to the funnel *A*, after replacing *B*, and the process repeated. Thorough and homogeneous mixing was found to have taken place when this had been performed six times. In this way the soil in large numbers of pots could be infected rapidly to about the same extent.

Three pounds of infected soil and one tuber were put into each pot. Watering was always effected by putting the water into the saucers and not directly on to the soil. It was found that when the soil was kept very wet during the first few weeks the sprouting tubers showed a tendency to decay. For the first two to four weeks, therefore, until the shoots appeared above the surface, the soil was kept fairly dry. For the remaining period, except when otherwise definitely stated, the soil was kept very wet by keeping water practically always standing in the saucers.

The time allowed between setting the tubers in infected soil and examining the plants for wart disease was generally about twelve weeks. This was found sufficient to permit of infection followed by considerable growth of warted tissue.

Relation of External Physical Conditions to Infection.

General field observation indicates that temperature and moisture are likely to play an important part in controlling infection of the potato plant by the wart disease organism. Temperature investigations carried out in America (6), using controlled soil temperature tanks, show that infection does not occur above 22° C., indicating a relatively low temperature requirement.

(a) *Moisture.*

The effect of varying moisture conditions on infection was studied empirically in the following way: soil, equally and homogeneously infected, was put into 40 pots and one Arran Chief tuber planted in each. The soil in 10 pots (Pots 21–30) was kept moist, rather wetter than was considered optimum for the potato plant. Daily inspection showed when these required watering, which was taken to be when the water standing in the saucers had been absorbed by the soil. Water was then applied

through the saucers to the soil in these 10 and the 30 other pots in the following quantities:

Pots	1–10 received	25 c.c.	per pot.
	11–20 ,,	50 ,,	,,
	21–30 ,,	100 ,,	,,
	31–40 ,,	200 ,,	,,

The soil was thus kept "dry," "fairly dry," "moist" and "very wet" in the different sets of pots.

Table I shows the number of plants infected in each set of ten. The absence of infection in the "dry" and "fairly dry" soils was not unexpected, but its complete absence in the "moist" soil was a little surprising. Heavy infection was however found in the "very wet" soil.

Table I.

Effect of Varying Soil Moisture on Infection[1].

Date of experiment: 25. vi. 22 to 2. x. 22. Variety of tuber: King Edward.

Pot numbers	Quantity of water per pot, per time watered c.c.	Condition of soil	Number of plants	
			Clean	Infected
1–10	25	"dry"	10	0
11–20	50	"fairly dry"	10	0
21–30	100	"moist"	9*	0
31–40	200	"very wet"	1*	8

* One out of the ten tubers planted failed to grow.

The absence of wart disease on plants grown in soil of a low or even moderate moisture content and its presence only under very wet conditions suggested a reason for many earlier failures to obtain a high percentage infection.

In 1922 potatoes grown in pots containing thoroughly infected soil in the glasshouse showed a low percentage infection (only 20 per cent.), while others set up in exactly the same way in the open had 85 per cent. of the plants well warted. In the former the soil was kept moist by moderate watering, while in the latter it was extremely wet because of the very heavy rainfall of that season. It thus appeared probable that insufficient soil moisture accounted for the low degree of infection obtained in the earlier glasshouse experiments. In the following year, therefore, pot experiments in the glasshouse were carried on in the same

[1] It may be interesting to note that infection by Rhizoctonia also increased with moisture. Practically none was found in the relatively dry soils of pots 1–20, while much Rhizoctonia was found on the plants grown in "moist" and "wet" soil (pots 21–40).

way as before except that the soil was kept very wet by having water continually standing in the saucers (after the first two or three weeks). In the open also, by means of abundant watering which was necessary in the dry season of 1923, the soil was maintained at a high degree of moisture. Heavy infection was found in 80–100 per cent. of the potato plants both in the glasshouse and out-of-doors. This was again the case in 1924. It would seem, therefore, that in previous experiments absence of sufficient soil moisture had been the limiting factor preventing infection.

The persistent appearance of an occasional clean plant under conditions which favour heavy infection in all its neighbours requires investigation. It is not impossible that the seed used, although carefully selected, may contain occasional immune individuals.

Again, 10 tubers were grown in one large pot (diameter 18 inches, height 12 inches) containing heavily infected soil kept moist in the glasshouse from December 20th, 1921, to May 1st, 1922. Growth was good but no sign of wart disease was found on any plant. The pot was moved out-of-doors and 10 new tubers planted in the same soil and grown from May 4th to September 13th, 1922, when every plant was found to be heavily infected. In the later case the soil was kept very wet by the heavy rainfall of the summer of 1922. At the same time it was thought that this result might be due to some possible "seasonal" effect which was further investigated, but, in the light of subsequent work described later in this paper, there can be little doubt that moisture was again the factor controlling infection. In subsequent experiments the soil was kept very wet and when other conditions favourable to the development of wart disease were maintained a high percentage, 80–100 per cent., of the plants were always infected. It thus appears probable that insufficient soil moisture is a common source of failure in pot experiments with wart disease.

The importance of moisture in controlling infection of potato plants by the wart disease organism, shown in the foregoing pot experiments, agrees with the common field observation that the incidence of the disease is much greater in wet seasons than in dry and that it is greater in the wetter regions, such as the northern and western counties of England than in the drier southern and eastern counties.

The very wet condition of the soil which was ascertained to be the factor determining infection by wart disease in potatoes grown in pots differs from that in the field in that it is continuous. Such a wet state of the soil exists under field conditions only at intervals during or soon

after rain. It therefore seemed possible that heavy infection, which is often found in the field, might also occur in pots when the soil was kept very wet for a part only instead of for practically the whole of the growth period. The following experiment was arranged to give some information on this question.

Seventy tubers were planted in separate pots containing freshly and uniformly infected soil. For the first four weeks all were treated alike and given, through the saucers, the same measured quantity of water which was only enough to maintain the soil in a "fairly dry" condition. At the end of this time shoots from all the tubers showed above the soil which could then be kept very wet without danger of the growth of the plants being prevented by rotting. From the fifth to the twelfth week watering was varied for each set of five pots. One set was kept "fairly dry" and two others "very wet" during the whole eight weeks, while the remaining sets severally were "very wet" for a month, a fortnight, a week, or two separate weeks, at different times. During the wet periods water was kept standing continuously in the saucers. These were emptied at the end of the wet week and no water was added for a fortnight, after which time the soil was "fairly dry." A small amount (100 c.c. per pot and once, in hot weather, 200 c.c.) was then given weekly. Except when the soil was "very wet" a "fairly dry" condition was maintained which aimed at being just wet enough to prevent wilting. The number of plants heavily infected, slightly infected, *i.e.* showing only minute traces of infection, and clean, are shown in Table II.

The five plants which were kept "fairly dry" during the whole period grew less well than the others but produced numerous small tubers quite sufficient to show infection if this had been present. The remaining plants were fairly well grown, having about four or five tubers per plant. No marked differences in the amount of subterranean growth were noted but the height of the aërial shoots varied considerably between the different sets of pots.

The variation in infection is shown in Table II. The plants grown in the "fairly dry" soil (pots 1–5) were all free from wart disease, while 10 plants grown in soil which was wet for the last eight weeks (pots 6–10 and 66–70) were all infected, most of them heavily. When the soil was wet for only one month, beginning at the fifth week (pots 11–15), all five plants were infected to almost the same extent as those which were wet for eight weeks. When, however, the wet period of four weeks began in the seventh and ninth week infection decreased to zero (pots 16–25). Again when the wet period was decreased to one and two weeks, infection

Table II.

Relation of Variation in Time of Wet Period to Infection.

Date: 25. i. 24 to 16. iv. 24. Variety: Arran Chief.

Pot Nos.	Condition of Soil during 12 weeks experiment — Week 1	2	3	4	5	6	7	8	9	10	11	12	Number of Plants: Heavily infected	Slightly infected	Clean
1– 5					-								0	0	5
6–10	–	–	–	–	W	W	W	W	W	W	W	W	5	0	0
11–15	–	–	–	–	W	W	W	W	–	–	–	–	5	0	0
16–20	-				–		W	W	W	W	–	–	0	3	2
21–25	-					-	--		W	W	W	W	0	0	5
26–30	–	–	–	–	W	W	–	–	–	–	–	–	2	1	2
31–35	–	–	–	–	–	–	W	W	–	–	–	–	0	0	5
36–40		-			-	-	–		W	W	–	–	0	0	5
41–45	–	–	–	–	W	–	–	–	W	–	–	–	4	1	0
46–50		–			W	–	–	–	–	–	–		1	2	2
51–55			--	--			W	–	–	–	–	–	0	1	4
56–60		-			–	–	–	–	W	–	–	–	0	0	5
61–65					–	–	-	–	–	–	W	–	0	0	5
66–70	–	–	–	–	W	W	W	W	W	W	W	W	4	1	0

– =fairly dry soil. W=soil kept very wet.

Table III.

Relation of Variation in Time of Wet Period to Infection.

Date: 22. ii. 24 to 13. vi. 24. Variety: Arran Chief.

Pot Nos.	Condition of Soil during 16 weeks experiment — Week 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Number of Plants: Well infected	Slightly infected	Clean
1– 5		–	-					-	–	-	-		-	–	-	–	0	0	5
6–10	–	-	–	-	W	W	W	W	W	W	W	W					5	0	0
11–15		-	–		W	W	W	W	-	–		–				-	4	0	1
16–20		–	–		-		W	W	W	W	–	--					4	1	0
21–25	–		–	–		–	–		W	W	W	W	–	-			2	3	0
26–30	-	–	–		W	W	–		–	–	–	–	–			-	4	0	1
31–35	-	-	-			–	W	W	--		–		–				1	0	4
36–40	–	–	–				-	-	W	W		–	-		-		1	2	2
41–45	--	--	–		W	–			W			-				-	2	3	0
46–50	–		–	-	W	-			-				–	-		-	0	0	5
51–55			–			–	W			--				-			0	2	3
56–60	-		–	-		-		-	W	-	-						0	0	5
61–65			–			–					W		–				0	0	5
66–70							--						-				0	0	5
71–75				–	W	W	W	W	W	W	W	W		-		-	1	0	1

– =fairly dry soil. W=soil kept very wet.

was slight when this period came in the fifth to the seventh week, and was entirely absent when it came later. Two wet weeks with a dry interval between (pots 41–45) resulted in infection which though slightly less than in pots 6–10 was fairly heavy.

These results again demonstrate the importance of moisture in controlling infection by wart disease of potatoes. They show that when the soil is kept wet for one period only this must last for about a month if it is to produce a high percentage of infected plants. Two wet periods of one week produced almost as much infection. Again, it is shown that a high degree of moisture during the middle period of growth is more effective in promoting infection than in the later period, but the significance of this result is not yet evident.

After these plants had been removed the same soil was kept "fairly dry" in the same pots for one month and then fresh tubers were planted, care being taken to avoid reinfection of one pot from another. When most of the shoots had appeared above the surface of the soil all the pots were kept very wet for two months, after which the plants were examined. Every plant was found to be well infected with wart disease. The capacity for infection was thus shown to be latent in the soil in every pot and to be controlled simply by moisture conditions. The earlier varied treatment does not appear to have materially affected the capacity for later infection.

A duplicate of the experiment whose results are shown in Table II, was set up one month after it had begun (Table III), and while the second experiment was still in progress the results of the first were obtained. The causes operative in producing so much greater infection when the wet period occurred in the second month than when it occurred in the third are not clear. The possibility was considered that when the wet period was in the third month infection might have taken place, but that the diseased growths might not have had time to develop before the plants were examined. In order to test this possibility the second set of pots, which up to the twelfth week was treated similarly to the first, was left for a further interval of four weeks, during which the soil in all the pots was kept fairly dry. The plants were then taken up and examined. In this way an extra month was allowed in which infected tissue might develop.

The results shown in Table III are in general similar to those obtained in the earlier experiment, but show a general tendency to greater infection. When the wet period of four or two weeks occurred in the third month (pots 21–25 and 36–40) some plants were found to be infected,

indicating that lack of time for development partially accounted for the complete absence of infection in similarly treated plants in the previous experiment (Table II, pots 21–25 and 36–40). The fact, however, that infection in pots 21–25 and 36–40, Table III, was less than in the previous experiment in pots 11–15 and 26–30, Table II, in which the same time was allowed for growth subsequent to the wet period may require further explanation. It is possible that in the second month the potato plant may be in a more susceptible stage of growth than it is four weeks later; it may, for example, be actively forming young shoots or tubers which in the period of rapid growth are readily infected. Or again, there may be some kind of periodicity in the germination of the sporangia which enables them to germinate more readily at certain times than at others. This point requires further investigation.

Moisture estimations made on the soils in pots 1–5 and 6–10 showed that the "dry" soil has a moisture content in the region of 5 per cent., and the "very wet" soil about 33 per cent.

From a practical standpoint these results show that a high degree of infection by wart disease can be obtained on potatoes growing in pots when the soil is kept very wet for the second month and fairly dry for the first and third months. There is also a distinct indication which requires further study, that if the soil is kept wet and dry alternately a high degree of infection may be obtained. These two results are of importance for soil sterilisation research in which it may be undesirable to keep the soil in a wet condition throughout the experiment because this may interfere with the normal reactions of chemicals in the soil.

(b) *Type of Soil.*

A second factor likely to influence the degree of infection is the physical character of the soil and therefore the following observations are of interest. A number of pots containing Arran Chief tubers were set up, using different types of equally infected soil. No significant differences could be detected in the amount of infection present (Table IV) and a high percentage of the plants grown in each type of soil were diseased. The heavy Rothamsted soil has long been recognised as too heavy to give satisfactory results in small pots without the admixture of some lighter material. When however this soil was used in a large pot, 12×18 inches, the resulting infection was as heavy as in the lighter soils. It thus appears that heavy infection can be obtained in soils varying in physical character from sand to a heavy field soil.

Table IV.

Numbers of Plants Infected in Different Types of Soil.

Date: June–Sept. 1923.

Soil	Number of plants		Percentage of plants infected
	Clean	Infected	
Sand	4	36	90
Light sandy soil from Ormskirk	1	9	90
Medium loam from Rothamsted allotments	3	17	85
Soil mixture consisting of field soil 37·5 % „ „ sand 50 % „ „ cavings 12·5 %	4	66	94

(c) *Seasonal Factors.*

The possibility that some "seasonal" factor or factors may be operative in controlling infection was suggested by certain results obtained in the earlier part of this work, the indications being of the following kind. Arran Chief tubers were planted ten at a time at monthly intervals from November, 1921, to March, 1922, each tuber being in a separate pot containing infected soil. The plants were grown in the glasshouse and the soil was kept moist but not very wet. Similar pots containing tubers planted in the same way from April to July, 1922, were put out of doors, where the unusually heavy rainfall of that season kept the soil extremely wet. After three or more months' growth the plants were examined and it was found that those planted from November to March and maintained in the glasshouse were only slightly infected, the numbers of plants showing signs of wart disease varying from none to four with an average of two out of each set of ten. Those planted from April to July and grown out of doors were, however, heavily infected, the disease appearing in at least eight out of each set of ten plants. These results suggested either a seasonal effect or the presence out-of-doors of some condition favourable to infection which was not operative in the glasshouse. The results set out in Table I, obtained while this series was in progress, showed that the presence or absence of a high degree of soil moisture might well be the controlling factor and explain what, superficially, might be interpreted as a seasonal effect. While the former appeared to be the more probable cause of variation in infection, the possibility of a seasonal factor being operative could not be ignored.

A series of monthly plantings were therefore carried out in 1923 and again in 1924. The sporangial material for use in the 1923 set was collected in the field during the summer and autumn of 1922, dried and

sieved all together and left exposed to light and air until required. In the same way the sporangia for use in 1924 were collected in the late summer of 1923 and treated similarly. Each month a measured quantity of this sporangial material was mixed with 30 lb. of soil and divided between ten pots in each of which one Arran Chief tuber was planted. After the first two or three weeks the soil was kept very wet for the remaining ten or nine weeks when the plants were examined. The pots were all kept in the glasshouse during the whole period.

The results obtained in 1923 and 1924 are shown in Table V. Heavy infection was found in every plant set up from December, 1922, to May, 1923, after which there was a slight (and so far unexplained) falling off in infection which may have been due to ageing of the sporangia. This hypothesis is supported by the fact that a higher percentage, 80–100 per cent., infection is obtained in the summer when new sporangia are used. The tubers planted after August, which were of the same stock as those planted earlier, were much shrivelled and made such poor growth that no reliable infection results could be obtained from them. No results were derived therefore from plantings in September, October or November. The results obtained in 1924 were similar to those of 1923.

Table V.

Variety: Arran Chief.

Date		Number of plants	
Month planted	Month examined	Clean	Infected with wart disease
Sporangia collected autumn 1922.			
21. xii. 22	15. iii. 23	0	10
18. i. 23	12. iv. 23	0	9
15. ii. 23	10. v. 23	0	10
15. iii. 23	7. vi. 23	0	10
12. iv. 23	5. vii. 23	0	10
10. v. 23	22. vii. 23	0	10
7. vi. 23	30. viii. 23	2	8
5. vii. 23	27. ix. 23	4	6
9. viii. 23	25. x. 23	1	9
29. viii. 23	22. xi. 23	4	6
Sporangia collected autumn 1923.			
17. i. 24	10. iv. 24	0	10
14. ii. 24	1. vii. 24	2	8
13. iii. 24	3. vii. 24	0	10
10. iv. 24	2. vii. 24	0	10
5. vi. 24	1. ix. 24	2	8
3. vii. 24	26. ix. 24	1	9

It thus appears that the apparent seasonal effect obtained in 1922 was simply dependent on variation in moisture. No evidence of any seasonal factor has been obtained and it therefore appears probable that a high degree of infection can be obtained in pot experiments of this type carried out at any season of the year.

The Winter Sporangium.

The fate of the winter sporangium in the soil is a problem of fundamental interest. It has been shown in the field that soil infected with wart disease retains, in the absence of the potato plant, its capacity for causing infection for at least nine years (5). The mode of survival of the parasite in the absence of the host is however obscure. It is known that *Solanum nigrum* and *S. dulcamara* are susceptible to attack by the fungus. It is possible that other weeds may act as hosts, thus enabling the organism to reinfect soil periodically with fresh sporangia. On the other hand, the sporangia may remain dormant in the soil until the presence of a host acts as a stimulus to germination, in which case the known longevity of such reproductive bodies in the fungi renders it not surprising that the soil should remain infected for many years. A further hypothesis is that the sporangia germinate irrespective of the presence or absence of a susceptible host. The germination may be spread over a number of years or the zoospores from the germinating sporangium may resort to a saprophytic mode of life resuming their parasitic propensities when suitable host plants are present. Curtis (2), however, observed that the zoospores after their discharge from the sporangium cannot survive long *in vitro*, and found no evidence of saprophytic tendencies. The whole question is of considerable importance and we have very little evidence bearing upon it.

(a) *Age of Sporangia and Infection.*

The survival in the soil of sporangia under the conditions of pot experiment for over a year is shown by the following observation. Sporangial material collected in the autumn of 1922 was kept dry until it was mixed with soil in the usual manner in March, 1923. The soil was put into pots in some of which tubers were planted immediately. After three months these were examined and found to be infected with wart disease. No tubers were put into the remaining pots but the soil was kept watered regularly and weeded periodically for a year. The soil was then allowed to become dry and remained so for some months. Tubers planted in these pots at the end of July, 1924, were examined

in October and found to be particularly heavily infected. The results are set out in Table VI. No diminution in the infecting power of the soil could be observed.

Table VI.

Survival of Winter Sporangia in the Soil.

Date		Number of plants	
Soil infected	Tubers planted	Clean	Infected with wart disease
16. iii. 23	16. iii. 23	0	10
16. iii. 23	31. vii. 24	0	9

Further evidence of the prolonged retention of vitality by the sporangium in the soil is shown by another observation made on sporangia collected in the summer of 1923. The sporangial material was kept dry until it was used to infect soil in October, 1923. The tubers were old and grew so poorly that only very slight infection was present. The soil was kept in the pots and replanted with tubers in July, 1924. On examination in October they were all found to be extremely heavily infected. The sporangia had thus remained in the soil in pots for about ten months with no apparent loss in vitality. It is however doubtful whether they retain their vitality so long when kept dry out of the soil. This point needs further investigation.

(b) *Dormancy in Winter Sporangia.*

A further point of some interest lies in the time necessary to obtain infection with winter sporangia. It has been observed in all the pot experiments hitherto carried out that no signs of infection appear until about two months after the soil has been infected and the tubers planted. Minute warts about $\frac{1}{8}$ inch in diameter are found at the end of this period and these by the end of another month have developed into large warted masses often one or more inches in diameter. The rapidity of growth suggests that the warts observed at the end of the second month are newly formed. It would appear therefore that either the sporangia lie dormant in the soil for a period before germinating and infecting the host, or that the potato plant is less liable to infection in the earlier stages of its growth.

The following test was arranged to throw some light on this question. Uniformly infected soil was put into 20 pots. Tubers were planted in only ten of these (pots 1–10), but all the 20 pots were equally well watered and treated similarly for one month. At the end of this time

tubers were planted in pots 11–20 and another set of ten pots (21–30) were filled with soil freshly infected and immediately planted with tubers. After a further period of one month all the potatoes in the whole of the 30 pots were examined. The results are shown in Table VII. The plants in pots 1–10, which had then grown for two months in the soil infected for the same period, showed small warts on nine out of ten plants. No infection appeared in the plants grown for one month in soil infected for one month (pots 21–30). The potato plants which had grown for one month in soil which had been infected for two months all had small warts on them (pots 11–20). These warted areas were about the same size as those obtained by infection from summer sporangia in about 14 days, so that infection may be assumed to have taken place within this period.

Table VII.

Dormancy of Sporangia.

Variety: Arran Chief.

Pot Nos.	Date: Soil infected	Date: Tuber planted	Date: Plants examined	Length of time since: Soil infected	Length of time since: Tuber infected	Number of plants: Clean	Number of plants: Infected with wart disease
1–10	16. iii. 23	16. iii. 23	11. v. 23	2 months	2 months	1	9
11–20	16. iii. 23	13. iv. 23	11. v. 23	2 „	1 month	0	10
20–30	13. iv. 23	13. iv. 23	11. v. 23	1 month	1 „	10	0

It thus seems clear that under these conditions the lapse of time before warts become evident depends, not on the stage of growth of the potato, but on the germination of the sporangium. There appears to be a definite time lapse in the region of six weeks after the sporangia are put into the soil before germination can take place. A similar but slightly longer delay in germination (ten weeks) was noted by Miss Curtis (2), dealing with sporangia *in vitro*. This lag in germination may depend on the time required for maturation of the sporangium, on the rate of penetration of the cell wall by water or on other less obvious factors, but the problem yet remains to be investigated.

(c) *Sporangial Numbers.*

Some knowledge of the relationship between the actual numbers of sporangia in the soil and the incidence of infection, is of considerable practical importance in pot experiments. In the following test carried out to throw some light upon this relationship, the sporangial material consisted of decayed warts and wart-bearing tubers, air dried, ground

down and passed through a sieve of 3 mm. mesh. Various quantities of the resulting powder were measured by volume and thoroughly mixed with 30 lb. of soil, which was then divided between ten pots in each of which one tuber was placed. An estimation of the actual numbers of sporangia per cubic centimetre of sporangial material was made as follows: The average weight of 1 c.c. of sporangial powder was found to be 0·825 gm. This quantity was prepared in a finely divided condition and the actual numbers of sporangia present estimated by the dilution method. This showed the numbers of sporangia present in 1 c.c. of dry sporangial powder to be in the region of 105,000. Table VIII shows the sporangial numbers added to 30 lb. of soil, and the numbers calculated per gram of soil.

Table VIII.

Relation of Numbers of Sporangia in the Soil to Infection.

Date: 12. iii. 23 to 4. vi. 23. Variety: Up-to-Date.

Volume of sporangial material. Mixed with 30 lb. soil	Numbers of sporangia	Numbers of sporangia per gram of soil	Number of plants	
			Clean	Infected with wart disease
200 c.c.	21,000,000	46,200	0	10
100 ,,	10,500,000	23,100	0	10
50 ,,	5,250,000	11,550	0	10
25 ,,	2,625,000	5,775	0	10
10 ,,	1,050,000	2,310	1	9
5 ,,	525,000	1,155	3	7
1 ,,	105,000	231	9	1

Variation in the quantity of sporangial material from 200 c.c. down to 25 c.c. produced no apparent diminution in the amount of infection, while from 25 c.c. down to 1 c.c. there was a rapid decline in the numbers of plants showing disease.

Relative Susceptibility of Different Varieties of Potato.

Great variation in the degree of susceptibility to wart disease has been observed in the field among different varieties of potato. It is of interest to learn, whether this difference in susceptibility is still evident under the more uniform conditions of pot experiment and, further, whether varieties which are only slightly susceptible in the field will show infection under these conditions. Ten different varieties of potato susceptible to wart disease were therefore grown in pots of heavily infected soil in the usual way. The varieties have been grouped by Mr H. Bryan, Superintendent of the Potato Testing Station, Ormskirk,

into those which in the field appear to be highly susceptible, moderately susceptible and slightly susceptible. A high percentage infection was obtained even in the least susceptible varieties grown in pots. The results are shown in Table IX and demonstrate that under conditions very favourable to infection a high percentage of even the least susceptible variety becomes warted.

Table IX.

Infection of Ten Different Susceptible Varieties of Potato grown in heavily infected Soil in Pots.

Date set up 17. i. 24.

Variety	Susceptibility in the field	Number of plants		Percentage plants infected	Average
		Clean	Infected		
Arran Chief	Great	0	19	95	90 %
President	,,	1	4	80	
Up-to-Date	,,	1	4	80	
Epicure	Moderate	0	5	100	87 %
May Queen	,,	0	5	100	
Ninety-fold	,,	2	3	60	
Sir John Llewelyn	,,	1	4	80	
Sharpe's Express	,,	1	4	80	
Puritan	,,	0	5	100	
British Queen	Slight	2	8	80	80 %

When however the soil was only slightly infected and the conditions were therefore less favourable to infection by wart disease, variation in degree of susceptibility became evident (Table X). The amount of infection was, in general, reduced and the incidence of disease was smallest in those varieties which proved least susceptible in the field. Thus under conditions which are not very favourable to infection, variation in the relative susceptibility of different varieties is evident and is found to be broadly similar to that observed in the field.

The possibility that under conditions which bring about a high percentage of infection in susceptible varieties immunity itself might break down, could not be overlooked. A further test was therefore carried out in which tubers of 12 immune and 12 susceptible varieties were grown in heavily infected soil under identical conditions. The results given in Table XI show that a high percentage of tubers belonging to susceptible varieties were infected, while all the immunes which on the whole showed better growth, and were therefore more liable to show disease, were free from infection.

From a practical point of view it is useful to know that in well-

Table X.

Infection of Ten Different Susceptible Varieties of Potato grown in slightly infected Soil.

Date: 12. vi. 23 to 19. ix. 23.

Variety	Susceptibility in the field	Number of plants		Percentage plants infected in each class
		Clean	Infected	
Arran Chief	Great	5	5	60 %
Up-to-Date	..	4	6	
King Edward	,,	1	4	
Epicure	Moderate	4	1	21 %
Duke of York	..	5	0	
Ninety-fold	..	3	1	
Sir John Llewelyn	..	4	1	
Sharpe's Express	..	3	2	
		4	1	
British Queen	Slight	5	0	10 %
Harbinger	,,	4	1	

Table XI.

Infection Experiments with Susceptible and Immune Varieties of Potato.

Date: 27. vi. 24 to 5. ix. 24.

Variety	Susceptibility under field conditions	Number of plants		Average
		Clean	Infected with wart disease	
Arran Chief	Great	0	10	93 %
King Edward	..	2	7	
President	,,	0	5	
Up-to-Date	..	0	5	
Epicure	Moderate	0	10	97 %
Queen Mary	..	0	5	
Ninety-fold	..	0	5	
Sir John Llewelyn	,,	0	5	
Sharpe's Express	,,	0	5	
Puritan	,,	1	7	
Harbinger	Slight	0	10	93 %
Mein's Early Round	,,	1	4	
Arran Comrade	Immune	10	0	0%
Roderick Dhu	,,	10	0	
Arran Comrade	,,	10	0	
Katie Glover	,,	10	0	
Tinwald Perfection	,,	10	0	
Golden Wonder	,,	10	0	
Di-Vernon	,,	10	0	
The Ally	,,	10	0	
Immune Ashleaf	,,	5	0	
Crusader	,,	5	0	
America	,,	5	0	
Majestic	,,	5	0	
The Bishop	,,	5	0	

infected soil, other conditions being favourable, infection of a high proportion of even slightly susceptible potatoes can be obtained in pot experiments, and that by this method immune varieties can be distinguished from susceptible ones. This test appears to be at least as reliable as field trials; it can be carried out more rapidly than such trials and does not depend for success on suitable seasonal conditions.

Alternative Hosts of *Synchytrium endobioticum*.

The possibility has been suggested that plants other than the potato act as hosts for *Synchytrium endobioticum* and thus help both to disseminate the disease and to prolong its existence in the soil in the absence of the potato plant. Cotton (1) has shown that *Solanum nigrum* and *S. dulcamara* are slightly susceptible to attacks by the fungus and Kunkel and Orton (4) that this is also true of certain varieties of tomato.

The infection of hosts other than the potato has here been attempted by the method of pot experiment described. The tomato plants were grown from seed sown in infected soil and then planted out in separate pots. The soil was heavily infected with *Synchytrium* sporangia and to facilitate infection, especially of the lower axillary shoots, the plants were set deeply so that several inches of the main stem were covered with soil. Macerated warts were mixed with water and poured round the stem while planting. Table XII shows the results of such tests obtained in 1922 with tomatoes and other plants of the family Solanaceae. Small warts measuring about 0·5 × 0·3 cm. in which winter sporangia were present were found on three plants of the variety Manx Marvel and one wart measuring 1·0 × 1·0 cm. on one plant of the variety Blaby. No trace of wart disease could be found on any of the other five varieties of tomato. Warts were also found on two out of four plants of *Solanum nigrum*, the largest measuring about 1·0 × 0·5 cm.

A similar test was carried out in 1923, the results of which are also shown in Table XII. Small warts, the largest measuring about 0·3 cm. diameter, were found on two varieties of tomato, Blaby and Fillbasket, and on four plants of *S. dulcamara*. The susceptibility of *S. nigrum* and *S. dulcamara* is thus confirmed and that of certain English varieties of tomato demonstrated. In all cases, however, the degree of susceptibility appears to be very slight.

In 1924, at the request of the Ministry of Agriculture and Fisheries, tests were carried out to ascertain whether certain plants belonging to the Solanaceae, which had been suspected of carrying wart disease, are susceptible to invasion by *Synchytrium endobioticum*. Extensive tests were made with *Salpiglossis sinuata*, *Atropa belladonna*, *Hyoscyamus*

niger and *Lycium chinense*, while control pots containing tubers of both the very susceptible variety Arran Chief and the slightly susceptible Harbinger were set up. The ten control Arran Chief plants were well infected and nine out of ten Harbingers. All the plants belonging to the four species tested were thoroughly examined but appeared to be entirely free from wart disease.

Finally it may be stated that during the carrying out of these pot experiments a considerable number of weeds have been examined for traces of infection by wart disease. Also during extensive field experiments extending over four years a close watch has been kept by Dr Brierley and myself on the weed flora of infected soil. Growths or appearances that suggested infection by wart disease were critically examined but in no case was wart disease found to be present.

It thus appears that slight infection by wart disease has been

Table XII.

Infection Experiments with Plants of the Family Solanaceae.

Name of plant	Date	Number of plants: Clean	Number of plants: Infected with wart disease
Tomato. Ailsa Craig	Planted May. Examined Oct. 1922	10	0
,, Blaby		9	1
,, Blako		8	0
,, Buckley		5	0
,, Comet		7	0
,, Fillbasket		5	0
,, Manx Marvel		5	3
Solanum nigrum		2	2
Datura Stramonium		5	0
Hyoscyamus niger		1	0
Tomato. Ailsa Craig	Planted June. Examined Oct. 1923	1	0
,, Blaby		8	1
,, Blako (Blaby × Kondine Red)		6	0
,, Buckley		6	0
,, Fillbasket		4	1
,, Improved Comet		5	0
,, Kondine Red		5	0
,, Manx Marvel		1	0
Solanum nigrum		5	0
,, *dulcamara*		1	4
Salpiglossis sinuata	Planted June. Examined Sept. 1924	25	0
Hyoscyamus niger		25	0
Atropa belladonna		25	0
Lycium chinense		25	0

observed in *Solanum nigrum* and *S. dulcamara*, and on certain varieties of tomato, all plants belonging to the family Solanaceae. Extensive investigation of other possible hosts apart from the potato has revealed no trace of wart disease.

INFECTION BY SUMMER SPORANGIA.

A consideration of the life-history of *Synchytrium endobioticum* suggests that infection by means of the summer sporangia, which germinate immediately, would be far more rapid than by the winter sporangia, which have a dormant period of many weeks before germination. When this occurs the winter sporangia liberate zoospores which infect growing potato tissue, resulting in a rapid development of sori of summer sporangia. These mature and in the presence of water are discharged, very soon liberating swarm-spores which either singly or after fusion in pairs infect fresh potato tissue, producing in the first case more summer sporangia, thus rapidly spreading the area of infection in adjacent potato tissue, or in the second case winter sporangia, which carry on the disease to the following season.

The inoculation of a clean growing shoot by means of newly discharged summer sporangia or by the zoospores set free from them seemed likely to provide a rapid method for producing infection.

Technique.

After some unsuccessful attempts to bring about infection by this means an easy and successful technique was devised which was carried out as follows: Fresh growing warts bearing summer sporangia were pinned on to tubers, in close contact with the young growing shoots to be infected. It was found to be of vital importance in obtaining infection that a film or drop of water should connect the warted tissue and the shoot to be infected practically all the time. The following simple apparatus was found to be effective. The tubers with attached warts are placed on a large piece of filter paper dipping into a vessel of water and supported over it by means of a wire frame. The tubers are well sprayed with water from a fine sprayer once or twice a day, are then covered with a sheet of damp filter paper and the whole covered with a bell-jar. In this way about a dozen tubers can be tested together. The infecting warts are renewed every seven days. By this means infection was obtained in most tubers, as described in the following experiments, in about 21 days. If the test is to be carried out on an extensive scale daily spraying can be avoided by the maintenance of an almost saturated

atmosphere around the tubers to be infected, *e.g.* by placing them after spraying under a bell-jar supported above a water bath kept at about 30° C. The tubers must be several (about 9″) inches above the water, otherwise the moisture tends to evaporate from them. In this way the wet film connecting wart and shoot need only be renewed by spraying about once in every few days.

EXPERIMENTAL.

Typical results obtained with susceptible varieties are shown in Table XIII. Summer sporangia were observed in many cases after 14 days. Occasionally in this work they have been detected after so short a period as seven days. After 21 days most tubers bore warts and after 28 days winter sporangia had developed, and could be detected on every susceptible tuber. No difference in degree of relative susceptibility of different varieties were observed.

Table XIII.

Infection of Susceptible Varieties by Summer Sporangia.

Date: 19. iii. 24 to 16. iv. 24.

Variety	Susceptibility to wart disease in the field	Total no. of tubers	No. tubers infected after 7 days	14 days	21 days	28 days
Arran Chief	Great	10	0	2	10	10
King Edward	,,	5	0	0	4	5
Up-to-Date	,,	5	0	4	5	5
Epicure	Moderate	3*	0	2	3	3
Sharpe's Express	,,	5	0	2	5	5
Ninety-fold	,,	5	0	3	5	5
Puritan	,,	10	0	3	10	10
Mein's Early Round	Slight	8*	0	1	8	8
Harbinger	,,	10	0	3	10	10
British Queen	,,	10	0	5	10	10

* Two out of the original five and ten tubers decayed.

If this method is to be of use in distinguishing immune from susceptible tubers, it must be shown that immunity does not break down under these conditions. The following trials were set up to test this question and Table XIV shows the results of infection experiments using both immune and susceptible varieties. After four weeks every susceptible tuber was infected with wart disease, while no signs of infection could be found on any of the immune varieties. Some of these were kept under treatment for another week but still showed no trace of infection.

Table XIV.

Inoculation of Susceptible and Immune Varieties by Summer Sporangia.

Date: 23. vi. 24 to 21. vii. 24.

Variety	Susceptibility to wart disease in the field	Total no. of tubers	No. of tubers infected after		
			14 days	21 days	28 days
Arran Chief	Great	4	0	4	4
King Edward	,,	5	0	5	5
President	,,	5	2	4	5
Up-to-Date	,,	5	0	1	5
Epicure	Moderate	5	0	4	5
Sir John Llewelyn	,,	4	1	1	4
Harbinger	Slight	3	2	2	3
Arran Comrade	Immune	5	0	0	0
Roderick Dhu	,,	5	0	0	0
Katie Glover	,,	5	0	0	0
Tinwald Perfection	,,	5	0	0	0
Golden Wonder	,,	5	0	0	0
Di-Vernon	,,	5	0	0	0
The Ally	,,	5	0	0	0
Immune Ashleaf	,,	4	0	0	0
Crusader	,,	5	0	0	0

CONCLUSIONS.

The need for a method of pot experimentation to serve as a basis for soil sterilisation research, which is now carried on almost wholly in the field and for a method of testing the immunity or susceptibility of new varieties of potato, has been indicated. The length of time involved in the field trials for immunity and susceptibility, which is at least two years, causes great delay in arriving at the results of genetic research and in putting new commercial varieties on the market.

The study of conditions controlling infection of potatoes by the winter sporangium of *Synchytrium endobioticum* in the soil in pots has shown that a high percentage of even the least susceptible varieties become infected when the right conditions are maintained. It is thus possible to carry out experiments with potatoes in small pots containing only about three pounds of heavily infected soil, and to obtain 80–100 per cent. of the plants infected. Soil moisture is a very important factor in controlling infection which is general and heavy only when the soil is very wet for a considerable part of the growth period. Pot experiments of this type can be completed in three months and can probably be carried out at almost any time of the year. They should therefore be useful in soil sterilisation and other research, and may be of use in testing for immunity.

A method of infection by summer sporangia has been devised which is capable of modification for use on an extensive scale for testing the immunity or susceptibility of new varieties. It can be carried out in a period of three or four weeks and apparently at any time of the year.

SUMMARY.

A study of certain conditions controlling infection of potatoes by the winter sporangium of *Synchytrium endobioticum* in the soil and by the summer sporangium in the laboratory has been made with a view to finding a reliable method of pot experiment to serve as a basis in soil sterilisation research, and a method for testing immunity or susceptibility more rapidly than is at present done in the field.

Experiments on infection by the winter sporangium in the soil have shown that a very high degree of soil moisture is necessary to ensure infection but this need not be present during the whole of the growth period. It appears most effective when the wet period is in the second month.

A high percentage infection is obtained in potato plants grown in soils of very varying physical character.

Under the conditions of pot experiment the wart disease organism survives in the soil in the absence of the potato plant for a period of at least a year. There appears to be a dormancy period of about six weeks between soil infection and sporangial germination. The relation of numbers of sporangia in the soil to the incidence of disease is discussed.

When favourable conditions were maintained 80–100 per cent. of the plants tested were found to be infected within a period of three months, even in varieties which in the field appear least susceptible. Under conditions less favourable to infection the relative susceptibilities of the several varieties become clearly marked. No wart disease was found under any conditions on immune varieties.

Infection of various plants other than the potato was attempted. Small warts were found on three varieties of tomato and on *Solanum nigrum* and *S. dulcamara*, but none on five other varieties of tomato, on *Datura Stramonium*, *Salpiglossis sinuata*, *Hyoscyamus niger*, *Atropa belladonna*, *Lycium chinense* or on many common weeds grown in infected soil.

A method is described for infecting sprouting tubers with wart disease by means of summer sporangia. Susceptible varieties subjected to this treatment develop young warts within three weeks, while immunes remain clean. The method can therefore be used for testing immunity or susceptibility in the laboratory.

I am indebted to Dr W. B. Brierley for much helpful criticism received during the course of this work.

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Addendum.

A paper by Spieckermann and Kotthof received while the present communication was in press, published in Germany in March, describes another method for testing immunity and susceptibility. A compost rich in germinating winter sporangia, prepared from warted material kept out of doors for several weeks in the autumn and preferably exposed to frost, was laid on the eyes of sprouting pieces of tuber and kept very wet at a temperature of 16–20° C. for about three weeks. Infection was observed in all susceptible varieties, and after six weeks a few varieties previously regarded as immune showed slight traces of infection.

Spieckermann, A. and Kotthof, P. *Die Prüfung von Kartoffeln auf Krebstigkeit. Deutsche landw. Presse*, 1924, li. 11, p. 114–115.

STUDIES ON CONTACT INSECTICIDES

PART I. INTRODUCTION AND METHODS.

PART II. A QUANTITATIVE EXAMINATION OF THE TOXICITY OF *TEPHROSIA VOGELII* HOOK. TO *APHIS RUMICIS* L. (THE BEAN APHIS).

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(With 3 Text-figures.)

During the last three years we have collected together a considerable mass of data with regard to the insecticidal properties of a number of chemical compounds and plant products. The material at our disposal will be best dealt with in sections and it is proposed to publish the results under the above heading, in two main divisions: (*a*) Plant Products, (*b*) Artificial and Synthetic Derivatives. By way of introduction and in order to avoid redundancy, we give in the first part of the present paper a brief outline of the objects of these investigations and some account of the general methods employed, whilst the second part of the paper deals with the insecticidal properties of the plant *Tephrosia Vogelii*, which seem to warrant special consideration.

Part I. Introduction and Methods.

At present the number of contact insecticides in common use is not very great. Nicotine is the most important, but an adequate alternative is a great desideratum. The search for such a substance forms the primary practical object of the investigation.

From the economic point of view, it is evident that intensity of toxicity is not the only important factor, but that price and supply are matters of equal significance. The continual improvements in chemical processes however render such factors as these exceedingly difficult to

evaluate, at all events for future guidance. It therefore became evident that the right method of approach to this problem was the more fundamental one—an endeavour to correlate chemical constitution and physical properties with toxicity to insects. With these objects in view, a somewhat extensive study has been attempted of a number of the more important groups of chemical compounds and naturally occurring plant products. It was hoped that in this way it might be possible to narrow the field of work and to select certain substances or groups of substances for more detailed and extensive study, whether from the practical or theoretical point of view. This stage has now been reached and publication of some of the results obtained seems justifiable.

Our intention being to test the insecticidal properties of a large range of compounds in this preliminary survey, it was necessary, on account of the increased labour and expense that would otherwise be involved, to limit our experiments for the most part to one species of insect. *Aphis rumicis* L. (the Bean Aphis) has proved very suitable for our purpose and the great majority of the experiments have been made with this species. With suitable care, this insect can be reared in large numbers on broad bean plants during the summer months and can be kept quite free from parasites. In order as far as possible to reduce individual variations, our stock has been reared each season from a single fundatrix and we have used only the adult apterous agamic females of each generation for the tests. (For further details see *Bull. Ent. Research* (1924), XIV, 223.)

In many cases the chemical compounds have been tested more than once and invariably at a number of different concentrations, ranging down in lethal effect, wherever possible, from strengths giving 100 per cent. of deaths to those giving no deaths at all[1]. By expressing the results in graphical form, we attempt to show in a simple quantitative way the comparative insecticidal intensities.

The apparatus and technique employed have already been dealt with by two of us in a paper on "An apparatus for testing the toxic values of contact insecticides (1)." It may be said that the spraying-machine there described has proved a very satisfactory instrument, enabling a large number of tests to be carried out very quickly under strictly controlled conditions. Special care has been taken in handling

[1] As stated by Tattersfield and Morris (1), the concentrations of poisons giving 50 per cent. of deaths are, from a theoretical and statistical point of view, the most suitable for a comparison of the toxic properties of the compounds. On the other hand, the concentration which will certainly secure 100 per cent. of deaths is the important one from a practical point of view and wherever possible this is given in both tables and diagrams.

the insects so as to reduce to a minimum the possibilities of injury. The removal of aphides from the plant while they are feeding cannot be done without risk of damage to the mouth-parts, but, by cutting the plants a few hours before they are required and allowing them to wilt slightly (in a large glass vessel) most of the insects wander off and can be picked up on a small brush.

We have generally sprayed 10 insects at a time for each individual test. The use of a larger number would have increased the accuracy of the figures by reducing the effect due to individual variations in resistance; but the period of the year during which the aphides can be reared in large numbers and normal healthy condition, is so short that the number of tests possible would have been seriously diminished, and for our present purpose this did not seem justifiable. The procedure adopted however renders it possible in most cases to make reliable deductions with respect to comparative toxicities and this was the immediate object. In order to minimise any effects upon the intensity of toxicity which may be due to changing meteorological conditions, we have, whenever possible, carried out tests with the more important compounds on several different dates.

In future work, with a limited number of selected compounds or groups of compounds, it will be necessary to make use of larger numbers of insects for each test and to compare toxicities on as many different types as possible.

Preparation of spraying mixtures.

The substances used have included both liquids and solids, soluble or insoluble in water. Liquids may either be dissolved in the spray medium or easily worked up into emulsions, whereas there is in general no little difficulty in working up solids insoluble in water into a state suitable for spraying. For example, a solid organic base insoluble in water may be dealt with in one of three ways: (1) by the use of an acid giving a salt soluble in water, (2) by dispersion in the spray fluid in an extremely fine suspensoid condition, (3) by dissolving in an organic solvent immiscible with water and working the solution up into the form of an emulsion. The first of these courses does not seem desirable as many of the organic bases are so weak that on the addition of water to their salts they decompose and the solution may become very acid, a fact which may materially affect the toxicity. The second course, probably the best in a fundamental sense, is very difficult as the solid particles tend to coalesce and to clog the spray nozzle and the toxicity is liable to be lowered through a corresponding decrease in the surface of contact.

These circumstances may give rise to abnormal results, an example of which occurred in one set of experiments with α-naphthylamine. In this case it was found that at a concentration of 1 per cent., very little toxicity was shown, but on dilution, toxicity increased to a certain point and then proceeded to decline in a normal way. This arose from the fact that the crystalline particles were large at the high strength and therefore only slightly effective; on diluting, the crystals became smaller owing to solubility and their effectiveness increased, while at a certain point the size of the particles reached a minimum and toxicity rose to a maximum.

In view of considerations such as these, it seemed best to adopt the third course referred to above, involving the use of an organic solvent. Benzene was chosen as the most suitable for the purpose. It has special advantages since our control experiments have shown that its toxicity to the insects used is not appreciable, and although almost completely immiscible with water it gives good emulsions on shaking with a 1 per cent. saponin solution. With a few difficultly-soluble substances it was found necessary to add small amounts of alcohol and ether to the benzene; in such cases special control tests with the mixtures of solvents were carried out.

For tests of this nature a wetting agent of some kind is obviously necessary. Saponin in 1 per cent. solution proved very satisfactory for this purpose and, in addition, it assisted emulsification. Control tests many times repeated showed it to be without toxic action to *Aphis rumicis*, a point which makes it much superior to soft soap for our purposes.

Method of expressing results.

Considerable experience has shown that, with aphides, examination for two days after spraying is generally adequate for reliable conclusions to be drawn. At each examination counts were made under four headings: "unaffected," "slightly affected," "moribund" and "dead," the greatest care being taken that unbiassed decisions were made. It would be possible to give numerical values or marks to each of these divisions, but such an evaluation would be somewhat arbitrary and in the tables and diagrams we have therefore included the "slightly affected" with the "unaffected," and also counted "moribund" and "dead" together. This method perhaps involves inadequate grading of comparative toxicities in some cases; but since most poisonous substances, when used at effective concentrations, cause an increase in the number of moribund and dead (or from moribund to dead) on the second day after spraying,

which would probably be followed by a further increase later, we believe that this method of treating the figures at all events does not exaggerate, though it may tend to minimise, toxicity.

A number of these compounds may also prove useful as stomach poisons, and it has been our practice to test the more hopeful among them in the following way. Hazel and hawthorn shoots have been sprayed with different concentrations of the material, allowed to dry and put into lamp-glasses fitted with one-holed corks, through which the stalk was passed into a test-tube of water. The top of the glass was closed by muslin held in place by a heavy iron ring. Caterpillars were then placed on the leaves in the jar and observed from day to day. After a few days the number of dead and alive insects were counted and observations made of the extent to which the leaves were eaten and of the degree of damage, if any, due to the chemical. An idea of the possibilities of the substance as a stomach poison and the tolerance of the plant to the chemical was thus obtained.

A systematic survey, such as that outlined above, should serve to bring out the relative toxic significance of a number of chemical compounds and groups substituted in them, and should thus act as a guide in the selection of suitable compounds both for further fundamental investigation and for trial on a practical scale. It is hoped in future to extend the work in both these directions.

We wish to express our great appreciation of the interest taken by Dr A. D. Imms throughout the course of this work.

Part II. A Quantitative Examination of the Toxicity of *Tephrosia Vogelii* Hook. to *Aphis rumicis* L. (the Bean Aphis).

In a paper on a "Quantitative Study of the Insecticidal Properties of *Derris elliptica*," by Fryer, Stenton, Tattersfield and Roach (2), it was mentioned that a certain number of insecticidal tests of a preliminary nature were carried out with the seeds of *Tephrosia Vogelii* Hook.; and it was found that these seeds had a measure of toxicity to silkworms. Owing to lack of material it was not possible at the time to carry out further tests, but later, through the good offices of Mr R. E. Massey, Botanist to the Sudan Government Department of Agriculture, a large specimen of the whole plant was placed at our disposal; and we wish to express our best thanks to Mr Massey for his material aid in this respect.

Experiments described here show that extracts of the leaves and seeds of this plant are highly toxic to our test subject, *Aphis rumicis* L., and although the investigation is not complete, we consider it advisable to set out the results so far obtained.

The genus *Tephrosia* (syn. *Cracca*) of the order Leguminosae is very widely distributed throughout the tropics of both hemispheres, occurring in South Africa, subtropical Australia and North America. Long lists of species are given by Bentham and Hooker (3), by Baker (4), by Welwitsch (5), and by Kuntze (6). Hooker (7) states that the genus seems peculiarly abundant towards the northern and drier parts of west tropical Africa, a large number of species being found in Senegambia. They range in size from herbaceous annuals and perennials through woody undershrubs to fairly large ornamental trees. Some of them find economic uses. *T. purpurea* would appear to have been cultivated and used for preparing a dyestuff, and in India for medicinal purposes. *T. candida*, and to a lesser extent *T. Vogelii*, have found application as green manures in many parts of the world; *T. Vogelii*, *T. densiflora* and *T. toxicaria* are utilised in the tropical countries of both hemispheres as fish-poisons.

According to Welwitsch (5), for use as a fish-poison, the leaves of *T. Vogelii* are bruised slightly and thrown into rivers, with the result that the fish are stupefied, come to the surface and are readily caught. Fish caught in this way are apparently quite wholesome.

T. Vogelii is a shrub 8–10 feet high, with woody branches covered with dense spreading foliage. The flowers are violet or rarely white, and the pods are 4 to 5 inches long and contain 16–18 seeds. It is found in West Africa, the Sudan, Rhodesia and the Comoro Islands, while it has

been experimentally cultivated for green manuring in the West Indies and in Java.

In addition to *T. Vogelii* other members of this genus, such as *T. toxicaria*, found native and cultivated in South America and the West Indies, and *T. densiflora*, closely allied to *Vogelii* and stated by Vogel to be cultivated in West Africa, as well as many other species, would probably be worth investigation as sources of insecticides.

The toxic principles of Tephrosia Vogelii.

The toxic principles of the leaves of *T. Vogelii* have been studied by M. Hanriot(8), who isolated three products: a yellow material crystallising in needles; a white crystalline compound, tephrosin; and a volatile substance with a strong aromatic odour which he names tephrosal.

He describes tephrosin as crystallising in small brilliant prisms, with a melting point of 187° C.; volatile with partial decomposition at high temperatures but capable of distillation *in vacuo* without chemical change, though suffering some decomposition on boiling with water. The pure compound is apparently insoluble in water, more soluble in acetone and dissolves completely in chloroform. Tephrosin contains no nitrogen and Hanriot ascribes to it the formula $C_{31}H_{26}O_{10}$. Tephrosal, to which the approximate formula $C_{10}H_{16}O$ is ascribed, begins to distil *in vacuo* about 60° C.; is only slightly soluble in water, partially soluble in alcohol and completely soluble in ether and chloroform. Its aqueous solution reduces ammoniacal silver nitrate in the cold and gives Schiff's reaction for aldehydes.

Hanriot found tephrosin to be extremely toxic to fish, roach being killed in one hour at a concentration of 1 in 25,000,000. All kinds of fish are sensitive but some less so than others, sea-water fish being on the whole more resistant. The same solution will kill numbers of fish introduced subsequently. Tephrosin is much less poisonous to other animals (frogs, crustacea, mollusca, worms, etc.). Dogs can tolerate a dose as large as 1 gm. given with their food, and rabbits can eat the leaves of the plant with impunity. Intravenous injection into dogs and rabbits brings on convulsions and paralysis; death is due to stoppage of respiration, a fatal dose for a rabbit being 0·01 gm. per kilo body weight.

The other two bodies isolated—tephrosal and the yellow substance —proved to be only slightly poisonous to fish, and the author considers it probable that this slight toxicity is really due to the presence of traces of tephrosin.

Hanriot's results were completely confirmed by work published in the *Bulletin of the Imperial Institute* for 1915(9). The three main con-

stituents were estimated and the leaves were found to contain: tephrosin 0·15 per cent., tephrosal 0·06 per cent., yellow substance 0·05 per cent. No alkaloidal or glucosidal substances were found and it was shown that the toxic properties to fish were mainly dependent on the tephrosin present. Examination of the seeds showed 12 per cent. of fixed oil and 0·3 per cent. tephrosin (M.P. 192° C. uncorr.), 0·11 per cent. of a new product melting at 158° C. and 0·008 per cent. of the yellow substance melting at 228–229° C. After the removal of these compounds by crystallisation there remained a considerable proportion of uncrystallisable residue, the composition of which it was not possible to determine. It is pointed out that as the seeds contain twice as much tephrosin as the leaves the assumption might be made that they would prove correspondingly more poisonous.

Experimental.

The leaves, the stems with leaves removed, and the seeds of the plant were examined for their toxicity to insects. The leaves and stems were derived from the Sudan, but the seeds obtained from this source were not in sufficient quantity to permit of quantitative tests being made. Use was therefore made of a supply of seed from the Gold Coast, which we had in our possession.

A finely-ground powder was used, extracted with alcohol by percolation in the cold (in the case of the leaves with ether and carbon tetrachloride also), the solvent used was partially evaporated on the water-bath, and finally in a vacuum desiccator. The extracts were taken up in the smallest possible quantity of benzene and made up to the required concentrations with 1 per cent. solution of saponin in water, and sprayed on to *A. rumicis* (adult apterous agamic females) in the Tattersfield-Morris spraying apparatus (1). The technique employed is referred to in Part I of this paper and also in *Bull. Ent. Res.* (1924), XIV, 223.

Many hundreds of insects were used in control experiments and, throughout the season's work, none were killed by the use of 1 per cent. saponin solution alone or with addition of benzene or alcohol in amounts considerably greater than those used to make up the test solutions. After spraying, the insects were set aside within easy reach of their food supply and examined over a period of two days. The results of the experiments with alcoholic extracts are set out in Tables I and II and graphed in Diagram 1, the percentage numbers of moribund and dead being plotted against the percentage concentration of dried extract used. In the second column of Table I and the third column of Table II are set forth the percentage of leaf, stem and seed equivalent to the

amount of extract used. These figures are calculated from the weights of extracted material obtained by cold percolation and not from those given by Soxhlet extraction.

The figures obtained by both methods of extraction are given in Table III. It will be observed that the weight of material extracted from the seeds by cold percolation with alcohol is considerably less than that obtained by using a Soxhlet apparatus, a result probably due to the relative insolubility of the fat in alcohol. In the case of the leaves and stems the two methods show little difference. Throughout this work extracts made by cold percolation were used in order to avoid complication due to possible chemical change in the toxic compounds at higher temperatures.

Table I.

Toxicity of Alcoholic Extracts of Leaves of Tephrosia Vogelii *to* Aphis rumicis (*adult apterous agamic females*).

Concentration					
% of dried extract	Equivalent % of leaves	Number of insects used	Number unaffected	Number moribund and dead	Per cent. moribund and dead
1st series.					
0·5	—	10	—	10	100
0·35	—	10	—	10	100
0·2	1·6	10	—	10	100
0·1	0·8	10	—	10	100
0·075	0·6	10	2	8	80
0·05	0·4	10	2	8	80
0·025	0·2	10	7	3	30
Controls	—	10	10	—	—
2nd series.					
0·3	2·4	10	—	10	100
0·2	1·6	10	2	8	80
0·1	0·8	10	4	6	60
0·075	0·6	10	7	3	30
0·05	0·4	10	7	3	30
Controls	—	10	10	—	—
3rd series.					
0·4	3·2	10	—	10	100
0·3	2·4	10	—	10	100
0·2	1·6	10	—	10	100
0·1	0·8	10	—	10	100
0·075	0·6	10	2	8	80
0·05	0·4	10	4	6	60
0·025	0·2	10	7	3	30
Controls	—	10	10	—	—

In all the above tests, including controls, 1 % of saponin was used as a wetting agent.

In Diagram 1 three sets of figures for the alcoholic extracts of the leaves, which are given in Table I, are plotted; these correspond to three separate series of tests and are marked X^1, X^2, X^3, respectively. The X^2 points show a lower toxicity than the other two sets. We con-

Table II.

Toxicity of Extracts of Leaves, Stems and Seeds of Tephrosia Vogelii *to* Aphis rumicis.

(Figures for nicotine are included for comparison.)

	Concentration		No. of insects used	No. unaffected	No. moribund and dead	Per cent. moribund and dead
	% of dried extract	Equivalent % of plant				
CCl_4 extract of leaves						
1st series.	0·5	—	9	—	9	100
	0·25	—	5	—	5	100
	0·1	—	8	2	6	75
2nd series.	0·5	—	10	—	10	100
	0·25	—	9	—	9	100
	0·1	—	10	3	7	70
Alcohol extract of stems	0·5	5·7	10	—	10	100
	0·35	4·0	10	—	10	100
	0·2	2·3	10	2	8	80
	0·1	1·15	10	6	4	40
	0·05	0·57	10	7	3	30
Alcohol extract of seeds	0·5	—	9	—	9	100
1st series.	0·35	—	10	—	10	100
	0·25	—	10	—	10	100
	0·1	—	10	—	10	100
	0·05	—	10	—	10	100
2nd series.	0·2	5·34	10	—	10	100
	0·1	2·67	10	—	10	100
	0·075	2·0	10	—	10	100
	0·05	1·33	10	1	9	90
	0·025	0·66	9	6	3	33
	0·01	0·27	9	7	2	22·2
Nicotine	0·08	—	49	1	48	98
	0·06	—	50	4	46	92
	0·04	—	50	20	30	60
	0·02	—	47	35	12	25·5
	0·01	—	50	47	3	6
	0·005	—	49	46	3	6·5
	0·0025	—	48	47	1	2

Table III.

Extracts of Tephrosia Vogelii.

(Per cent.)

	Leaves		Stems		Seeds	
Solvent	Soxhlet	Percolation in the cold	Soxhlet	Percolation in the cold	Soxhlet	Percolation in the cold
Alcohol	13·7	12·4*	8·41	8·8*	14·15	3·75*
Ether	7·2	—	2·84	—	12·76	—
Carbon tetrachloride	7·4	—	—	—	—	—
Benzene	7·7	—	—	—	—	—

* These are the figures for the extracts used in the tests.

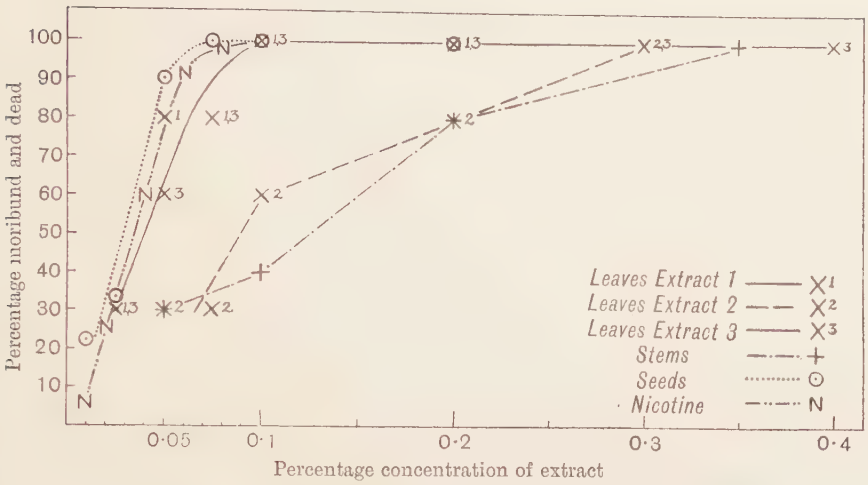

Diagram 1. Showing toxicities of alcoholic extracts of *T. Vogelii*.

sider that this probably arises from the fact that on diluting benzene solutions of the dried extract with 1 per cent. saponin solution there is a tendency for it to separate out from the solvent in a solid form and for the particles to coalesce. It is therefore possible that in certain cases the effective concentration may be rather lower than that expressed. This point is raised as having a bearing upon actual spraying practice as it might tend to lower the value of such extracts. This difficulty did not arise with extracts of the seeds which are easily worked up into an emulsion and have not so far presented any difficulty in spraying.

The extracts of leaves and seeds have proved themselves highly toxic to aphides, their toxicity being of the same order as that of nicotine, with which comparison is made in Diagram 1, where the most toxic results obtained by us for that substance are expressed (see Table II). The standard of comparison is therefore as high as possible. Inspection of Table II and Diagram 1 shows, however, that the extracts of the stems are not so toxic as those of the leaves and seeds.

The fact that the concentrated alcohol extract has a toxicity of the same order as that of pure nicotine indicates that the active principle itself must be an excessively toxic body. We have had no opportunity of isolating Hanriot's tephrosin, but there is little reason to doubt that this compound, proved by him to be extremely toxic to fish, is also responsible for the results obtained by us with Aphides.

Aqueous Extracts. As the dried extracts of leaves and stems obtained by the use of organic solvents present a little difficulty in working up

into emulsions suitable for spraying, it was decided to test spray fluids obtained by simple aqueous extractions. For this purpose weighed quantities of the ground leaves, stems and seeds were allowed to stand for a little time with distilled water. The mixtures were then filtered

Table IV.

Toxicity of Aqueous Extracts of Tephrosia Vogelii *to* Aphis rumicis.

	Concentration per cent. of plant	Number of insects used	Number unaffected	Number moribund and dead	Per cent. moribund and dead
Extract of leaves + 1 % saponin	2·5	10	—	10	100
	1·0	10	—	10	100
	0·5	10	4	6	60
	0·25	10	5	5	50
	0·1	10	9	1	10
Extract of leaves (water only)	2·0	8	1	7	87
	1·0	10	5	5	50
	0·75	10	7	3	30
	0·5	10	8	2	20
	0·25	10	10	—	—
	0·1	10	6	4	40
The lower toxicity shown in this series is probably due to imperfect wetting of the insects.					
Extract of leaves + 0·5 % soft soap	2·0	10	—	10	100
	1·0	10	—	10	100
	0·75	10	—	10	100
	0·5	10	—	10	100
	0·25	10	—	10	100
The soft soap used is itself toxic to *A. rumicis* and probably accounted for 50 % of the deaths.					
Extract of stems + 1 % saponin	10·0	10	4	6	60
	5·0	10	7	3	30
	2·5	10	10	—	—
	1·0	10	8	2	20
	0·5	10	10	—	—
	0·25	10	9	1	10
Extract of seeds + 1 % saponin	5·0	10	—	10	100
	2·5	10	—	10	100
	1·0	10	4	6	60
	0·5	10	7	3	30
	0·25	10	9	1	10
Extract of seeds (water only)	2·0	10	—	10	100
	1·0	10	—	10	100
	0·75	10	—	10	100
	0·5	10	10	—	—
	0·25	10	9	1	10
There was an appreciable amount of solid matter suspended in this extract.					
Extract of seeds + 0·5 % soft soap	1·0	10	—	10	100
	0·75	10	—	10	100
	0·5	9	—	9	100
	0·25	10	—	10	100
See note on soft soap above.					

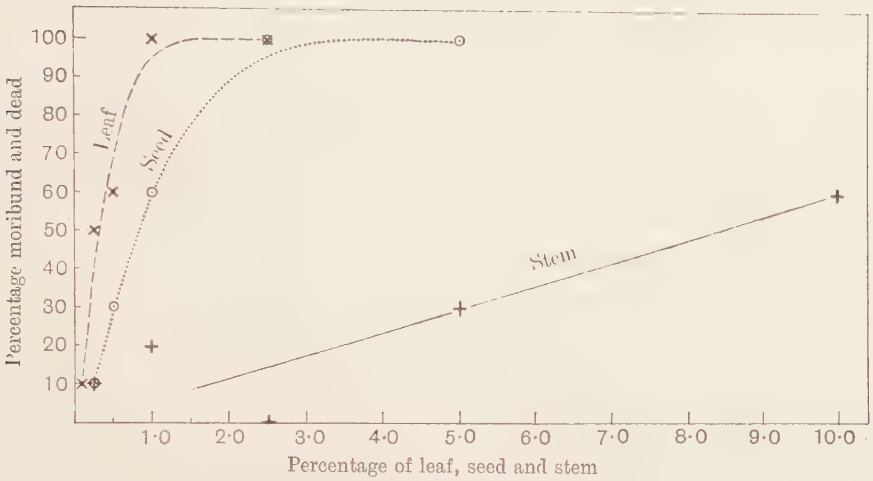

Diagram 2. Showing toxicities of aqueous extracts of *T. Vogelii.*

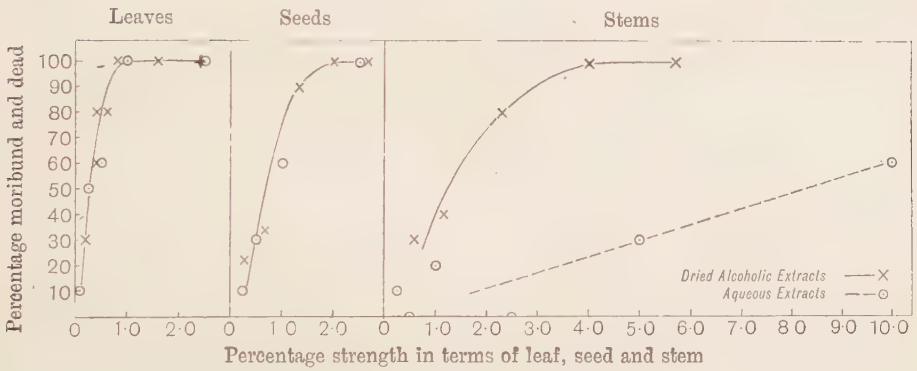

Diagram 3. Showing comparative toxicities of aqueous and alcoholic extracts of *T. Vogelii.*

through fine muslin, the filtrates being put through several times until clear of any particles large enough to clog the spray nozzle. Saponin was then added in a quantity sufficient to bring its content up to 1·0 per cent. and dilutions made with 1 per cent. saponin solution. The solutions were sprayed in the usual way.

The results are shown in Table IV and Diagram 2.

The aqueous extracts of the leaf and seed proved to be of a highly poisonous nature to *A. rumicis*, while those of the stems were only toxic in high concentrations, the toxicity in descending order being (1) leaves, (2) seed, (3) stems.

In order to compare the toxicities of aqueous and alcoholic extracts

on the same basis, we have calculated the weights of extracts obtained by cold percolation with alcohol to the equivalents of their respective parts of the plant (leaf, seed, stem). These values shown in the second column of Table I and the third column of Table II, are plotted on Diagram 3 against the percentage of moribund and dead insects, the points being marked by a $\times$. The points marked $\odot$ are the figures for the aqueous extracts set out in Table IV. In the cases of leaf and seed there is a very close agreement between the results. There is thus a clear indication that water alone will take up the available poison almost completely, a matter of some surprise since the extracts with organic solvents are almost insoluble in water. It is significant that Hanriot points out that aqueous extracts of the leaves are much more toxic to fish than would be expected from the slight solubility of tephrosin in water.

The aqueous extracts of the stems are less toxic than those prepared by alcohol percolation from the same weight of material (Diagram 3). This may be due to mechanical difficulties or some protection from extraction afforded by the presence in the stem of material not easily wetted or insoluble in water.

Table IV also contains data of spraying tests made with aqueous extracts of the leaves and seeds to which no saponin was added and some in which the wetting material used was soft soap, at a concentration of 0·5 per cent.

Where no saponin was added to the leaf extract the results show a toxicity lower than those given when it was present. Some form of wetting material would appear necessary in practical use. In the case of the seeds, aqueous extracts without saponin proved completely toxic at as low a dilution as 0·75 per cent. of the seeds. The concentrated mixture applied in this particular case was prepared of as thick a consistency as could be drawn through the nozzle in the spraying machine and it may be that some of the effect is mechanical. The results are therefore not strictly comparable with the others discussed, but confirm the high toxicity of the seeds.

Table IV also shows the results obtained with aqueous extracts when soft soap was used as a wetter. The soft soap used was itself toxic to these insects, but it was desirable to put through a few tests as it seemed possible that the poisons might be affected in an adverse way by this material. This was found not to be the case and soft soap may safely be used with aqueous extracts. In these experiments, controls showed that probably about 50 per cent. of the deaths might be accounted for by the soap alone.

Coincident with these small scale trials carried out in the spraying machine, a few spraying tests were made on a normally occurring heavy infestation of bean plants in pots, 0·5 per cent. of soft soap being used to secure adequate wetting.

The results were as follows:

Extract equivalent to	2 %	of leaf:	5 unaffected,	3 moribund, 505 dead.
„ „	1 %	„ :	8 „	remainder dead (some hundreds).
„ „	0·5 %	„ :		All dead („ „).
„ „	2 %	of seed :		„ („ „).
„ „	1 %	„ :		„ („ „).

Some tests were also carried out to see whether this material acted as a stomach poison to caterpillars. Springs of hazel were sprayed with various dilutions of the aqueous extracts, allowed to dry and larvae of the moth (*Selenia tetralunaria* Hufn.) placed upon them. (For details of method see p. 65.) The results of this experiment were not very conclusive, the foliage being rendered so repellent that the larvae for the most part refused to feed. This experiment, however, served to demonstrate that the application of these extracts had no deleterious action on the foliage.

Conclusions.

Both alcoholic and aqueous extracts of *Tephrosia Vogelii* Hook., a plant which is found both wild and cultivated in many parts of the tropics, have been found to have a high toxicity as a contact poison to *Aphis rumicis* L. The toxic effect of the concentrated extracts on this insect is of about the same order as that of nicotine. The possibility has not been lost sight of that this high toxicity may be to some extent specific towards the type of insect used for the tests, and further work on other insects is contemplated.

The poisons themselves have not been isolated by us; they are, however, most probably those which give to this plant its intensely poisonous properties to fish, and which have already been isolated and to some extent examined by chemists. The toxic substances apparently occur only in small quantities in the plant. It is, however, obvious from our experiments that if this plant proves to have any wide range of application as an insecticide it will not be necessary to attempt to isolate these substances. Aqueous extracts of the leaves[1] and seed proved very

[1] Since this work was complete, one reference has been noticed by us with respect to the toxic properties of *T. Vogelii* to insects. W. M. Aders (*Zanzibar Protectorate Ann. Rep. Publ. Health Dept.* 1916, 32–37) showed that mosquito larvae placed in a 1 per cent. solution of pulp made from leaves died in 12 hours.

toxic when a suitable wetting reagent was used with them. In practice it would appear that aqueous extracts of the ground leaves would suffice for most purposes, and our experiments indicate that they would be preferable to those made with organic solvents; the latter on drying tend to become somewhat insoluble and the aqueous extracts have about the same toxicity. The aqueous and alcoholic extracts of a given weight of seed also have an equal toxicity, but in this case the dried extracts prepared by means of organic solvents are more readily worked up into an emulsified condition. They can easily be taken up by some solvent and form a good emulsion when shaken up with water containing soap or saponin. The stems are less poisonous than either leaves or seeds.

Summary.

1. The first part of this paper forms a general introduction to investigations carried out on contact insecticides. The methods used are briefly described and some consideration is given to the mode of expressing results.

2. The second part deals in detail with the insecticidal properties of *Tephrosia Vogelii* Hook. which, with other species of this genus, occurs abundantly in many parts of the world. The aqueous and alcoholic extracts of the leaves and seeds of *T. Vogelii* are shown to be highly toxic to *Aphis rumicis* L., the toxicity being of the same order as that of nicotine. Extracts of the stems have not proved so poisonous.

3. The plants of the genus *Tephrosia* seem to offer possibilities for practical use as insecticides and it is hoped to extend the work in this direction.

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FURTHER EXPERIMENTS IN THE CONTROL OF CERTAIN MAGGOTS ATTACKING THE ROOTS OF VEGETABLES

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Introduction.

The trials discussed in the following article are a continuation of experiments begun in 1920 and carried on each season since, so far as the vagaries of climate and incidence of insect attack would allow. The work in question commenced with attempts at the control of the root feeding maggots of the onion and carrot flies, to which have been added (1924) some preliminary experiments with the cabbage root maggot and the turnip gall weevil.

The four insects, then, concerned in these trials are:

Hylemyia antiqua Meigen. The onion fly.
Psila rosae Fabr. The carrot fly.
Chortophila brassicae Bouche. The cabbage root fly.
Ceuthorrhynchus pleurostigma Marsh. The turnip gall weevil.

The life-histories of all four are somewhat similar, the larval stage in each case being spent in the roots of the various vegetables. It is in this stage that so much damage is done to the crops concerned. The host plants in the case of the first two insects are indicated by their names and the depredations of the two latter are directed to various Cruciferous crops. In Cheshire and Lancashire, where this work has been carried out, there is no doubt that the three flies mentioned constitute the worst insect menace with which the market-gardeners and small-holders have to deal.

Owing to the sheltered existence of the maggots, doubly protected by soil and root tissue, it is exceedingly difficult to attack them successfully, and because of this mode of life attention in methods of control has been largely directed to the use of chemicals deterrent to the fly, rather than to poisons to kill the maggot.

The insects will be discussed in what appears to be their order of economic importance.

Hylemyia antiqua Meigen. The Onion Fly.

The following brief description of the appearance and habits of this fly is taken from an earlier paper of the writer's (1).

> The fly is a grey insect somewhat like the housefly in appearance though rather lighter in colour. Its body is about 6 millimetres long and measures half an inch across the wings. The thorax is a lighter grey than the rest of the body and has a number of large bristles interspersed with small ones running longitudinally giving the thorax a banded appearance. The abdomen is darker than the thorax and is much more heavily set with black bristles. In the male the eyes are very closely set together. The female is very similar to the male in general appearance, except that it is rather lighter in colour, the eyes are widely separated and the abdomen is broader and pointed at the end owing to the presence of the ovipositor....The egg is white and about 1 millimetre in length, it is deposited in clusters usually under the thin sheathing leaf surrounding the stem of the onion, or in the crutch formed by the outside leaf and the stem.
>
> The maggot on hatching from the egg, makes its way through the soil and enters the onion through the base of the bulb.

The flies are on the wing early in May and egg laying may continue till the middle of September. As will be seen from these trials, the maggots were still feeding when the onions were harvested at the end of September.

In choosing chemicals for trial as deterrents for the onion and other flies, the two main points to bear in mind are: Firstly, ease of application, for example in the trials under discussion, the chemicals had to be such as could be applied in powder form, as it would have been impracticable to obtain water for their application, and this must often be the case where onions are grown in quantity. The second point is cheapness, these two points are obviously closely connected. Before proceeding to the current year's experiments, it will be worth while to review shortly the methods and chemicals used heretofore in this and other countries for the control of the onion fly. Practically all entomologists abroad who have experimented in the control of this insect have confined themselves to the use of the "Poison Bait." This is a mixture of molasses and sodium arsenite and has been used both as a spray and as a trap placed about the field in various receptacles.

Eyer (2) describes his experiments with this bait. He also used carbolic acid emulsion, but came to the conclusion that this was too expensive to be of any commercial value. In addition he tried sowing carbolated lime and calcium hypochlorite with the seed, at the rate of

two ounces to the rod, with some success. He found dry lime sulphur a failure.

Tawse(3) used the poison bait method and also recommends the planting of trap crop onions.

Ruhmann(4) gives the following conclusions: "Three years' experiments show that poison baits, though causing high mortality, do not materially prevent oviposition. Trap crops take the major portion of oviposition of the first generation during the most critical period of the growth of the seedlings. This treatment is not expensive and has proved successful under commercial conditions." It is not clear whether the trap crops consist of autumn sown onions. In this country it has been often pointed out that autumn sown onions, or onions sown in heat, are less liable to attack than the spring sown crop (Roebuck(5)).

The following is a list of chemicals, together with the concentration and the method of application, used by the writer(6) on the experimental plots in Cheshire during the seasons prior to 1924.

(1) Green tar oil. 1 per cent. mixture in sand. Later in chalk.
(2) Para-di-chlor-benzene. In sand, half an ounce per square yard.
(3) Potassium permanganate. In water. 1 ounce in 2 gallons.
(4) Naphthalene and soap snuff. As powder, ¾ ounce per square yard.
(5) Light cresylic acid. In sand. 1 per cent. mixture.
(6) Heavy cresylic acid. In sand. 1 per cent. mixture.
(7) Light creosote. In sand. 1 per cent. mixture.
(8) Heavy creosote. In sand. 1 per cent. mixture.
(9) Nicotine. In water. 1 ounce in 5 gallons.
(10) Soot. Dusted uniformly over the soil surface.
(11) Soft soap and sulphur. In water as a spray. 2 ounces flowers of sulphur and 1 ounce of soap in every 2 gallons of water.
(12) Rosin soap. In water. 1 ounce per 2 gallons.
(13) Sodium sulphocarbonate. In water. 1 ounce per gallon.
(14) Carbolic acid emulsion. In water as a spray. Crude carbolic acid, 1 pint. Hard soap, 1 lb. Water, 1 gallon. Used at the rate of 1 part to 35 parts water.
(15) Soap and cresylic acid. Soft soap mixed as an emulsion with the cresylic acid and sand. Soft soap 1 ounce per 10 square yards. Cresylic acid 1 per cent.
(16) Soot and derris powder. 1 ounce derris, 2 ounces soot, per square yard.
(17) Nicotine dust and soap powder. 10 parts of an equal mixture to 10 parts of precipitated chalk.
(18) Ortho-cresol. 1 to 5 per cent. in chalk.
(19) Paraffin emulsion. Paraffin, 4 pints. Soft soap, 1½ lb. Water, 10 gallons.
(20) Chlor-cresylic acid. 1 per cent. in sand, latterly chalk.
(21) B. naphthylamine. 2 per cent. in chalk.
(22) Nitro benzene. 2 to 5 per cent. in chalk.
(23) Aniline. 2 to 5 per cent. in chalk.
(24) Corrosive sublimate. 1 ounce in 10 gallons water.
(25) Nitre.

Of these chemicals, green tar oil, chlor-cresylic acid, nitro-benzene, nicotine dust and soap powder, nicotine (in water), and ortho cresol were the ones which showed promise, and some of these were given continuation trials this season (1924).

In 1922 and 1923 the plots were set out and treated with various chemicals as usual, but owing to illness in one case and lack of fly infection in the other, no results were forthcoming for those two years.

From the experiences of trials of past years, it has been noticed that an attack of *H. antiqua* upon a field of onions tends always to be of a patchy and uneven nature, and except in cases of infection of unusual severity, such as that experienced in 1920, the onions are rarely uniformly affected. Perhaps the two greatest factors influencing attack, apart from weather conditions, are firstly the presence of shade trees and secondly the texture of the soil.

In the field used for these trials there are a number of sycamore and elm trees at one end, which throw a considerable amount of shade upon the onions growing in their vicinity. Each year it has been observed that infection in these particular plots is double that in the other plots. This may be due either to some preference on the part of the fly for shade or to a possible deleterious effect of the shade upon the health of the onions, thus leaving them more liable to attack by the fly. As regards the effect of soil texture, attack always appears greater in loose light soil than in a heavy clay. Bearing these points in mind, it was decided at the commencement of these trials to have a control plot of exactly similar nature to each treated plot instead of only one or two check plots for the whole field as hitherto.

The field was divided up into eleven treated plots and eleven check plots. Each one was 20 square yards in area, the two series of treated and untreated plots ran parallel the length of the field and were divided by a narrow path.

The chemicals were applied at intervals of ten days, beginning at the end of May and continuing till the 8th of September, about ten applications in all.

In reviewing the results of these trials, the abnormal weather conditions of the summer of 1924 must be considered. The deterrent effect of each chemical must have been greatly reduced by the long-continued and torrential rain.

The variety of seed sown was "Giant of Zittau," an onion greatly in favour in this particular district.

Each chemical was applied as a powder spray, the "carrier" or

Results of Onion Trials of 1924.

Plot No.	Chemicals used	% strength in chalk	No. of Applications	Total weight of onions	Weight at time of harvesting		Percentage
					Clean	Infected	
				(lb.)	(lb.)	(lb.)	
1.	Creosote	1	10	68	53	15	77 clean 23 infected
2.	Nicotine sulphate	5	10	111	92	19	82 clean 18 infected
3.	Ortho-cresol	1	10	121	86	35	71 clean 29 infected
4.	Chlor-cresylic	1	10	147	107	40	72 clean 28 infected
5.	Anthracene oil	5	10	145	90	55	62 clean 38 infected
6.	Ortho-cresol carbonate	5	10	163	133	30	81 clean 19 infected
7.	Para-cresol carbonate	5	10	148	120	28	81 clean 19 infected
8.	Ortho-nitro-ortho-cresol	5	10	165	128	37	77 clean 23 infected
9.	Tetra-chlor-ethane	5	10	147	110	37	74 clean 26 infected
10.	Nitro-benzene	2	10	106	80	26	75 clean 25 infected
11.	Meta-cresol carbonate	5	10	112	92	20	82 clean 18 infected

Results from Onion Check Plots, 1924, *compared.*

Plot No.	Total weight of onions (lb.)	Weight of clean onions (lb.)	Weight of infected onions (lb.)	Percentage	Weight of clean onions compared	
					From trial plot (lb.)	From check plot (lb.)
1.	66	45	21	68 clean 32 infected	53	45
2.	95	47	48	49 clean 51 infected	92	47
3.	111	66	45	55 clean 45 infected	86	66
4.	145	75	70	51 clean 49 infected	107	75
5.	144	84	60	58 clean 42 infected	90	84
6.	130	87	43	67 clean 33 infected	133	87
7.	133	92	41	69 clean 31 infected	120	92
8.	120	80	40	66 clean 34 infected	129	80
9.	110	70	40	63 clean 37 infected	110	70
10.	70	55	15	78 clean 22 infected	80	55
11.	98	76	22	77 clean 23 infected	92	76

"filler" used being precipitated chalk. The strengths of the mixtures are appended. The sprays were applied by a small powder sprayer which was worked by a hand bellows and alternatively a metal sprinkler was used. Both were satisfactory, though the hand bellows were found to be rather slow and tiring for use over a wide area.

The following tables give the chemicals used with the respective strengths on application and the results obtained.

Discussion of the Results achieved by the 1924 *Onion Trials.*

In securing results from treating such a crop as the onion, it is difficult to arrive at a method of compilation which takes into consideration the large numbers of plants which have been destroyed since the commencement of the fly infection in spring. It is manifestly impossible to count all the plants, as could be done in experiments with the cabbage, for example. The only satisfactory method appears to be that of weighing the total harvest from each plot and comparing the result with the harvest from the corresponding untreated plot. That is the procedure followed here, the weight of clean and maggoted onions being given in each case, together with the percentages, the latter of course only referring to the maggots present at the actual time of harvesting. This method of counting results is a very slow and arduous one where there are many plots to be considered, but it has been followed here in default of a better or more accurate procedure. As will be seen from the foregoing tables, a true estimation of these results can only be formed by a comparison of each plot with its particular check. Plots 1 and 2, with their very reduced total yield due to the shade before mentioned, should not be compared with plots 8 and 9, which were grown under much more favourable conditions on heavy soil.

Plot 1. *Creosote.* A yield of only 53 lb. uninfected onions as compared with 45 lb. on the check plot. In 1920 creosote gave only 25 per cent. clean onions, and in 1921 only 5 lb. more than the check plot. This is therefore the third trial of creosote as a preventive of onion fly, and results do not justify its further use. It appears more efficacious in experiments against cabbage root fly (7).

Plot 2. *Nicotine sulphate dust.* A yield of 92 lb. as compared with 47 lb. This is the most successful of any of the chemicals used in these trials. In 1920 nicotine used in water gave 68 per cent. clean onions, and used as a dust in combination with soap powder in 1921 it produced a yield of 47 lb. as compared with 32 lb. on the check plot. The nicotine sulphate shows an improvement on these earlier experiments. It is

cheaper than nicotine and with chalk, as a 5 per cent. mixture, it is easy to apply.

PLOT 3. *Ortho-cresol.* This gives an increased yield of 20 lb. over the control. In 1921 the increase was 8 lb. This substance shows some promise but requires further testing.

PLOT 4. *Chlor-cresylic acid.* This plot shows a superiority of 32 lb., a yield of 107 as compared with 75 lb. In 1921 the superiority of yield over the control was 28 lb. Chlor-cresylic acid can be recommended for use in onion fly control. It is economical to use, only very small quantities being necessary. Its chief drawback is its exceedingly penetrating and clinging odour. Care should be taken not to exceed in the mixture a strength of 1 per cent.

PLOT 5. *Anthracene oil.* Yields 90 lb. of clean onions compared with 84 lb. on the check plot. Shows little promise and requires further testing. It has, like creosote, apparently been fairly successful in experiments against cabbage root fly.

PLOT 6. *Ortho-cresol-carbonate.* 133 lb. compared to a yield of 87 lb. from the check plot. Used, like the para- and meta-cresol carbonates, for the first time. Further tests are necessary.

PLOT 7. *Para-cresol-carbonate.* Yields 28 lb. more than the control. Needs further testing.

PLOT 8. *Ortho-nitro-ortho-cresol.* Gives 125 lb. as compared with 80 lb. from the check. This promises well, but in all probability will prove too expensive for general use as an insecticide.

PLOT 9. *Tetra-chlorethane.* Gives 40 lb. more than the control. Used for the first time in 1924 against onion fly. Gives encouraging results, but is too volatile to be of much use in the field.

PLOT 10. *Nitro-benzene.* 80 lb., compared to 55 lb. on the check. This gave good results in the trials of 1921, but is inferior to chlor-cresylic acid.

PLOT 11. *Meta-cresol-carbonate.* 92 lb. to 76 lb. control. Results very similar to those obtained with para- and ortho-cresol carbonates.

PSILA ROSAE Fabr. THE CARROT FLY.

The next experiments to be discussed are those dealing with the carrot fly, second only in importance to the onion fly. *Psila rosae* is a small black shining insect with iridescent wings. Its body is one-sixth of an inch long and has a wing expanse of half-an-inch. The head is dark brown in colour, and the legs are pale yellow (6). The appearance of this insect is quite different from that of the onion fly, though the habits

and life-history of the two are somewhat similar, there are, however, as a rule, only two generations of the carrot fly in the season.

The trials of 1924 with this insect are disappointing. The infection of the carrots did not take place until very late in the season, in fact not until early August, the usual time of the appearance of the second generation. When the fly did appear, however, the infection was widespread and severe. In these trials, as in the others, the heavy rainfall largely interfered with the insecticidal effect of the chemicals used. The applications made, *i.e.* once a fortnight, were apparently too infrequent under the circumstances.

There were six trial plots and one control plot. The area of each bed being approximately 30 square yards, each plot was divided from its neighbour by a path about 2 feet wide.

Of the chemicals used in these trials only one, creosote, had been tested in previous years.

The following substances were used, and their method and strength of application are appended. The seed was sown on March 22nd, 1924.

Nicotine sulphate dust. Applied in a 5 per cent. mixture with chalk.
Tetra-chlorethane. Applied in a 5 per cent. mixture with chalk.
Creosote. Applied in a 1 per cent. mixture with chalk.
Para-cresol-carbonate. From 5 to 10 per cent. in chalk.
Meta-cresol-carbonate. From 5 to 10 per cent. in chalk.
Ortho-cresol-carbonate. From 5 to 10 per cent. in chalk.

The results are as follows:

Plot 1. Para-cresol-carbonate.
Clean carrots ... 44 %
Infected „ ... 56 „
Plot 2. Creosote.
Clean carrots ... 57 %
Infected „ ... 43 „
Plot 3. Ortho-cresol-carbonate.
Clean carrots ... 40 %
Infected „ ... 60 „
Plot 4. Tetra-chlorethane.
Clean carrots ... 45 %
Infected „ ... 55 „
Plot 5. Meta-cresol-carbonate.
Clean carrots ... 33 %
Infected „ ... 67 „
Plot 6. Nicotine sulphate.
Clean carrots ... 65 %
Infected „ ... 35 „
Plot 7. Control.
Clean carrots ... 30 %
Infected „ ... 70 „

Discussion of Results.

Of these six substances tried only those on Plots 2 and 6 showed any sign of real control. Nicotine sulphate dust was again the best, giving 65 per cent. good roots.

Creosote, which has given promising results in previous trials, yielded a percentage of 57 per cent. clean carrots. The other plots all

yielded under 50 per cent. marketable carrots and showed little improvement upon the control plot. These substances require further testing under more normal conditions.

CHORTOPHILA BRASSICAE Bouche. THE CABBAGE ROOT FLY.

This insect is very similar in appearance and life-history to *Hylemyia antiqua*, the onion fly. The following description is taken from a paper on the cabbage root fly by Gibson and Treherne(8):

> The fly is about one-quarter of an inch in length from the tip of the head to the end of the abdomen, the body and legs being of a dark ash-grey colour, and rather thickly covered with black bristles. On the back of the thorax are three blackish rather broad bands, the central one of which continues along the back of the abdomen. The eyes, which nearly touch each other in the male, are of a reddish purple colour. At the base of the femur of each hind leg there is a conspicuous cluster of rather long bristles, and this is a character which is used to separate the male of this species from the male of closely-allied species, such as the onion fly.

The eggs are first laid in spring upon the stem of the plant or the surface of the soil near by, and oviposition continues throughout the summer. There are probably three generations of the fly in the season. The food plants of the maggots are the roots of various members of the Cruciferae, the most commonly attacked being cauliflower, cabbage and turnip, and occasionally radish and rape.

The experiments outlined herewith are preliminary and the results obtained do no more than indicate possible lines for future trial. These experiments are divided into two parts, the first consists of a series of field trials, designed to test the efficacy of various chemicals. The second is a series of pot experiments confined entirely to the use of corrosive sublimate in various strengths, in order to determine whether the claims put forward for it in the U.S.A. and elsewhere, as a certain cure for cabbage root maggot, are justified. The field trials were carried out as follows. Nine chemicals were chosen for trial, some of which have been used with success elsewhere. In a field of cabbages alternate rows of 50 were used for the employment of the chemicals. The cabbages between were untouched, two rows at the other side of the field being used as controls.

The following are the chemicals and concentrations used:

Chlor-cresylic acid	1 % in chalk.	Ortho-nitro-ortho-cresol	5 % in chalk.
Nitro-benzene	5 „ „	Green tar oil	1 „ „
Ortho-cresol	1 „ „	Creosote	1 „ „
Tetra-chlorethane	5 „ „	Nicotine sulphate dust	5 „ „
Anthracene oil	2 „ „		

They were applied as a powder mixed with the chalk and scattered round the stem of the plant at fortnightly intervals from the time of setting out.

The following are the results of these preliminary trials; it will be seen that the infection, even in the control plots, was not such as to give the chemicals a fair test.

1st 50 *cabbages.* Treated with nitro-benzene.

Number infected	10	Percentage infected	20
Number clean	40	Percentage clean	80

2nd 50 *cabbages.* Tetra-chlorethane.

Number infected	12	Percentage infected	24
Number clean	38	Percentage clean	76

3rd 50 *cabbages.* Ortho-nitro-ortho-cresol.

Number infected	11	Percentage infected	22
Number clean	39	Percentage clean	78

4th 50 *cabbages.* Green tar oil.

Number infected	2	Percentage infected	4
Number clean	48	Percentage clean	96

5th 50 *cabbages.* Anthracene oil.

Number infected	5	Percentage infected	10
Number clean	45	Percentage clean	90

6th 50 *cabbages.* Chlor-cresylic acid.

Number infected	0	Percentage infected	0
Number clean	50	Percentage clean	100

7th 50 *cabbages.* Ortho-cresol.

Number infected	5	Percentage infected	10
Number clean	45	Percentage clean	90

8th 50 *cabbages.* Creosote.

Number infected	4	Percentage infected	8
Number clean	46	Percentage clean	92

9th 50 *cabbages.* Nicotine sulphate dust.

Number infected	7	Percentage infected	14
Number clean	43	Percentage clean	86

10th 50 *cabbages.* Control.

Number infected	12	Percentage infected	24
Number clean	38	Percentage clean	76

11th 50 *cabbages.* Control

Number infected	15	Percentage infected	30
Number clean	35	Percentage clean	70

The best results from these trials were obtained from the use of chlor-cresylic acid, which gave 50 clean plants, next come green tar oil and creosote. Anthracene oil and nicotine sulphate dust also promise well. Brittain(7) records good results from the use of creosote and

anthracene oil, but does not consider nicotine sulphate worthy of further trial. It is hoped to give the above-mentioned four chemicals a further trial in an ensuing season.

The next set of experiments in connection with *C. brassicae* deals with the use of corrosive sublimate (mercuric bichloride). This substance has been so largely used and recommended in the United States of America and elsewhere, as a remedy for the attacks of cabbage root maggot, that it was considered worth while to try it out under control conditions. Forty seedling plants of cauliflowers (variety Autumn Giant) and the same number of cabbage seedlings (variety Utility) were planted in large pots which were sunk in the ground level with the surface. The pots were then divided up into four series, each series having four experiments, there being five plants used in each experiment.

The five plants (cauliflower and cabbage in each case) were treated as follows: six half-grown maggots were introduced into four of the five pots, the fifth plant being left uninfected.

The first two plants in each experiment were infected with six maggots and treated with bi-weekly doses of corrosive sublimate, the third plant had corrosive sublimate only and the fourth and fifth had six maggots only and acted as controls. The complete set of experiments then was as follows:

SERIES A. *Cauliflowers.* Strength of corrosive sublimate used: one part in 1000. Four experiments of five plants each.

SERIES B. The same, using cabbages.

SERIES C. *Cauliflowers.* Strength of corrosive sublimate used: one part in 500. Four experiments of five plants each.

SERIES D. The same, using cabbages.

The object of growing the plants in pots was to prevent migration and possible reinfection by the maggots. The sublimate was applied in sufficient quantity to soak the roots, and several applications were made at intervals of from four to five days.

The following results were obtained:

SERIES A. *Cauliflowers.* Strength used, 1 in 1000.

(*a*), (*b*) are plants infected and treated with corrosive sublimate.
(*c*) plant treated with corrosive sublimate only.
(*d*), (*e*) plants infected with maggots, but otherwise untreated.

Exp. 1. (*a*) Recovered } maggots pupated normally.
(*b*) Recovered }
(*c*) Died from external infection.
(*d*) Recovered.
(*e*) Recovered, but died later from a secondary external infection.

Exp. 2. (*a*) Recovered.
(*b*) Died.
(*c*) Remained healthy.
(*d*) Died.
(*e*) Died.

Exp. 3. (*a*) Recovered.
(*b*) Recovered.
(*c*) Healthy.
(*d*) Died.
(*e*) Died.

Exp. 4. (*a*) Recovered.
(*b*) Recovered.
(*c*) Healthy.
(*d*) Died.
(*e*) Died.

SERIES B. *Cabbage*. Strength used, 1 in 1000.

Exp. 1. (*a*) Recovered.
(*b*) Recovered.
(*c*) Remained healthy.
(*d*) Died.
(*e*) Died.

Exp. 2. (*a*) Recovered } maggots pupated
(*b*) Recovered } normally
(*c*) External infection.
(*d*) Died.
(*e*) Died.

Exp. 3. (*a*) Recovered.
(*b*) Recovered.
(*c*) Remained healthy.
(*d*) Recovered.
(*e*) Recovered.

Exp. 4. (*a*) Recovered.
(*b*) Recovered.
(*c*) Remained healthy.
(*d*) Recovered.
(*e*) Recovered.

There were only two experiments possible in Series C and D owing to lack of material.

SERIES C. *Cauliflower*. Strength used, 1 in 500.

Exp. 1. (*a*) Died } maggots pupating
(*b*) Died } normally.
(*c*) Remained healthy.
(*d*) Died.
(*e*) Died.

Exp. 2. (*a*) Recovered.
(*b*) Died.
(*c*) Externally infected.
(*d*) Died.
(*e*) Died.

SERIES D. *Cabbage*. Strength used, 1 in 500.

Exp. 1. (*a*) Recovered } maggots pupated
(*b*) Recovered } normally.
(*c*) Healthy.
(*d*) Died.
(*e*) Died.

Exp. 2. (*a*) Recovered } maggots pupated
(*b*) Recovered } normally.
(*c*) Healthy.
(*d*) Recovered.
(*e*) Recovered.

A further test which was made of the effect of corrosive sublimate in solution on the unprotected maggot is here shortly described. Owing to the lack of sufficient cabbage root maggots, the larvae of the onion fly were used for this experiment. Six full-grown maggots were immersed for three minutes in each of the following strengths of corrosive sublimate in water:

(*a*) 1 part in 1500 parts of water.
(*b*) 1 „ 1000 „ „
(*c*) 1 „ 500 „ „
(*d*) 1 part in 100 parts of water.
(*e*) 1 „ 20 „ „

One each in cases (*c*) and (*e*) died, the rest were apparently unaffected. These preliminary experiments with corrosive sublimate, though not

conclusive, yet appear to cast considerable doubt upon the efficacy of this substance as a preventive of cabbage root maggot. In no case was it found that the maggots at the roots of the plant were killed or their pupation prevented by the applications(11). Although it is true many of the plants so treated recovered, yet it is equally true that many of the control plants did likewise, more so in the case of the cabbage than in that of the cauliflower. This recovery was largely due to the pupation of the maggots and the production of secondary rootlets by the plant above the place of injury.

It may be claimed that the sublimate has not been fairly tested as a preventative of oviposition or as a possible ovicide. This may be so, but it should also be noted that in three cases the control plant treated with the sublimate only became infected through external agency. In these three cases at all events the sublimate did not prevent oviposition. There is no doubt that these tests were made under conditions adverse to any insecticide, owing to the continuous heavy rainfall of the season of 1924, and the corrosive sublimate should be given a further test before it is entirely discredited as a remedy for cabbage root maggot.

CEUTHORRHYNCHUS PLEUROSTIGMA Marsh. THE TURNIP GALL WEEVIL.

The following summary of the life-history of this beetle is taken from the work of Isaac(9):

> The cause of the smooth round galls on the roots of cultivated crucifers is the presence of the larvae of *Ceuthorrhynchus pleurostigma*. This pest has a wide distribution over Europe and is common all over the British Isles. The egg is laid in the bark on the root, the larva lives in the gall thrown out by the plant, and the pupa is found in the soil. There are two distinct races of this insect, a spring race which breeds in *Sinapis arvensis* and not at all in cultivated crucifers, and a summer race which breeds in *Brassica campestris* and *B. oleracea*. The spring race does not injure crops.

The adult beetle is a small black shining insect, with a long rostrum, at the middle of which are inserted the antennae. Curtis(10) describes this insect fully.

At a conference held by the Ministry of Agriculture early this year (1924), the question was raised by Mr A. Roebuck, a member of the conference, as to whether the galls produced by the weevil on the cabbage really affected the health of the plant, or if the cabbage did not finally outgrow the effect of the gall and produce an ordinary marketable head. As an outcome of this discussion comparative trials of galled and ungalled cabbage crops were arranged in various parts of England and Wales, and the following is a description of one such trial carried out in Cheshire.

The variety Ox cabbage of a late kind was used, 400 plants, 200 galled and 200 healthy, were planted in two blocks divided by a path in rather heavy loam just outside Manchester. No farmyard manure was given but two applications of nitrate of soda were made. All the plants were grown under exactly similar conditions in similar soil. In each block there were eight rows of 23 cabbages and one of 16. The cabbages were planted out on May 12th, 1924. At the time of planting the two sets of plants were easily distinguishable from each other. The galled cabbages were smaller and of a purplish tint compared with the larger and greener ungalled plants. The following are the results obtained from this trial:

Ungalled Plot. 200 cabbages planted.

Total number of heads harvested = 172.
Total weight of 20 best heads = 198 lb.
Average weight of best heads = 9·9 lb.

Galled Plot. 200 cabbages planted.

Total number of heads harvested = 165.
Total weight of 20 best heads = 195 lb.
Average weight of best heads = 9·75 lb.

Conclusions.

As will be seen, in each case the number of cabbages harvested is somewhat below that of the original 200 planted. These losses were due solely to the attacks of slugs and cabbage root maggot, especially the former. The slugs appeared particularly attracted by the galls and probably for this reason the number of plants killed by the slugs was greater in the case of the galled cabbages.

The final harvest from the plots is very similar and there was little to choose between the appearance of each plot at the end of the season. The cabbages were all healthy, and equally fine heads were harvested from both lots.

The deductions to be drawn then from this test appear to be two. One is the apparent attraction of the galls for the slugs, and the second and more important is the fact that the health of the cabbage in the seedling stage is impaired. As already mentioned, at the time of setting out the plants, the two sets showed a marked difference in appearance, the galled seedlings being much less healthy.

In this particular test the cabbages were planted under ideal conditions and two or three days steady rain succeeded the setting out. It

appears likely that had the weather been hot and sultry at the time of planting many of the galled seedlings might have succumbed.

It will be of interest to compare these results with those of similar trials held in other parts of the country.

SUMMARY.

Experiments dealing with four species of root feeding maggots are described. The insects concerned are: *Hylemyia antiqua*, the onion fly; *Psila rosae*, the carrot fly; *Chortophila brassicae*, the cabbage root fly; and *Ceuthorrhynchus pleurostigma*, the turnip gall weevil.

Control experiments on the first two have been carried on since 1920. A list of all the chemicals used in these trials is given.

As a result of the 1924 experiments the following are recommended for use against onion fly: (*a*) nicotine sulphate dust, in a 5 per cent. mixture with precipitated chalk; (*b*) chlor-cresylic acid, in a 1 per cent. mixture with precipitated chalk.

After three seasons' trials creosote is not considered an efficient remedy for onion fly.

For carrot fly: (*a*) nicotine sulphate dust in a 5 per cent. mixture; and (*b*) creosote, in a 1 per cent. mixture, both in chalk, gave promising results in the 1924 trials.

Of chemicals used in field trials against the cabbage root maggot the most successful were: (*a*) chlor-cresylic acid, (*b*) green tar oil, (*c*) creosote. Anthracene oil and nicotine sulphate also gave fair results.

A number of pot experiments, designed to test the efficacy of corrosive sublimate as a control for cabbage root maggot, were carried out. Results do not appear to justify the use of this substance as a remedy, but further trial is necessary.

400 cabbages, 200 galled by the larvae of the turnip gall weevil and 200 healthy, were grown under test conditions in order to ascertain the effect of these galls upon the health of the plant. The results of this experiment indicate that firstly the galls are attractive to slugs and secondly that the critical time in the life of a galled cabbage is the seedling stage, but that given good cultural conditions at the time of setting out, the cabbage will flourish normally.

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THE FERN MITE. (*TARSONEMUS TEPIDARIORUM*, WARBURTON)

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(With Plates III–V and 5 Text-figures.)

Introduction.

While visiting various nurseries in Kent my attention was directed to a disease of ferns, popularly called "Fern Mite Disease," the cause of which was rather obscure. The growers had experienced a great deal of trouble in the cultivation of the valuable greenhouse fern—*Asplenium bulbiferum*—since the disease was so difficult to control. The damage to the plants was so considerable, that the growers were thinking of giving up the cultivation of this particular fern (Pl. III, fig. 6).

On examining the foliage, especially the younger fronds, both curled and uncurled, it was found to be infested with a mite, which was identified as *Tarsonemus tepidariorum*, Warburton, by Mr S. Hirst of the British Museum.

At the suggestion of Professor H. M. Lefroy I undertook the following investigations.

History of the Species.

Adults of the species *Tarsonemus tepidariorum* were first described by Warburton (31) in 1904. In his report he says:

> In September last some injured fern fronds were received from a nursery with inquiries about the "fern mite" which, it is alleged, had made its appearance about two years ago and had since become so serious a pest that some nurserymen had been obliged to give up the fern-growing part of their business. Mites were found upon the under surface of the leaves sent, chiefly congregated in the neighbourhood of the veins, and all stages were present, from the eggs to the mature males and females. They were not, however, clustered together in large numbers, as in the case of the gall mites.

There appear to be no other records of this particular mite either in Europe or America, but the disease appears to have been present for some years in England, although only recently has it become serious in certain districts, notably around London.

Tarsonemus tepidariorum, being the specific cause of this disease in ferns, the name of fern mite seems very appropriate.

Systematic Relationship.

The family *Tarsonemidae*, to which this mite belongs, is one of much biological and economic importance. According to Banks (6), they are soft-bodied mites, and in some ways resemble the *Tyroglyphidae*, but the females differ from these, as well as from all other Acarina, in having between legs I and II on each side, a prominent clavate organ of uncertain use. They are very small mites, averaging about 1/120 of an inch in length, and the males and females vary greatly in appearance, and might easily be taken for entirely different species. The integument of the female appears to show segmentation, which is quite unusual in the Acarina.

The family is divided into two sub-families, *Pediculoidinae* and *Tarsoneminae*. The sub-family *Tarsoneminae*, containing two genera[1], is distinguished by the fact that the hind legs in the female end in long hairs, and those in the male are about as long as the third pair of legs. The two genera, *Scutacarus* and *Tarsonemus*, are represented by a considerable number of species, and many of the species of the latter genus are of distinct economic importance. The characteristic features of the genus *Tarsonemus*, are that the body is elongate, the mouth-parts are terminal, and that the fourth pair of legs in the female are extremely thin and weak and end in two long bristles, while in the male they are strong clasping organs, not used for locomotion at all, but dragged behind the animal as it moves along.

The original name of the genus, created by Canestrini and Fanzago (11) in 1876, was *Chironemus*, which included some soft-bodied mites found in colonies on leaves. The name, being preoccupied, the authors changed it the following year to *Tarsonemus*.

Food Plants.

As far as is known, *Asplenium bulbiferum* and its many varieties (Pl. III, fig. 7) appear to be the only host plants of this pest.

Occurrence on the Plant.

Since the mites habitually shun the light they are found in the innermost recesses of the unopened fronds and pinnae, on the incurled upper-

[1] Ewing has just created a new genus—Locustacarus—for a new species of mites taken from the tracheae of certain American grasshoppers. *Proc. Ent. Soc. Washington*, xxvi, No. 3, March, 1924.

side of the young fronds, which are just unfolding, among the scale leaves and scales at the base of the plant, and also on the young offspring plants situated on the old fronds. They have also been found on the under surface of the newly unfolded fronds. The mites show a preference for any hollows or grooves, either on the pinnae, or on the stalk. They are found in all stages of development, on the younger parts of the plants, but never on the older leaves. The distorted appearance (Pl. V, fig. 10) which some of the latter show is, therefore, due to the attacks of the parasites when these fronds were young. As the frond gets older it gets less succulent and the epidermis gets evidently too tough and leathery to allow the mouth-parts of the mite to pierce it. This point is proved by the fact that, when mites, both adults and larvae, were put in cells in which was also placed a piece of leaf from an old frond, all the larvae and most of the adults died within a day, the remainder surviving a few days. This did not happen when a young leaf was placed with the mites under the same moist conditions. Also, since these Acarina in all stages require a certain degree of humidity, without which they would shrivel up and die, we may suppose that another reason why the older parts of the plants are immune to their attacks, is because of the lack of moisture in these parts.

The mites seem to be able to walk a considerable distance on the plant, and also, probably, on the surface of the soil, provided there is a sufficiency of moisture. There is every reason to believe the latter to be the case, for, though they have not actually been observed on the ground, they are capable of walking some distance on a moistened glass surface.

Description of *Tarsonemus tepidariorum*.

The Egg. The egg, though very small, is large considering the size of the adult. It is oval, and white in colour, possessing a pearly appearance which does not change as the embryo develops as some mite eggs do. The resultant larva is pure white in colour. The egg has rather a delicate, granulated shell, which has no constrictions; and sometimes eggs may be found collapsed. The egg measures about 113·4μ in length and about 75·6μ in breadth.

The Larva. The larva (Fig. 4) is pale, glistening white. There are three pairs of legs, two of which are anterior and point forwards, while the third pair are posterior and point backwards. The legs are very short and each is composed of five free parts terminating in a pulvillus and a blunt bifid claw. The claws are used on a rough surface, while the pulvilli serve as excellent adhering organs on smooth surfaces.

On the dorsal side the skin is soft, wrinkled and elastic generally, but there appear two areas, one near the anterior and one near the posterior end of the body, which are free from these striations. As the

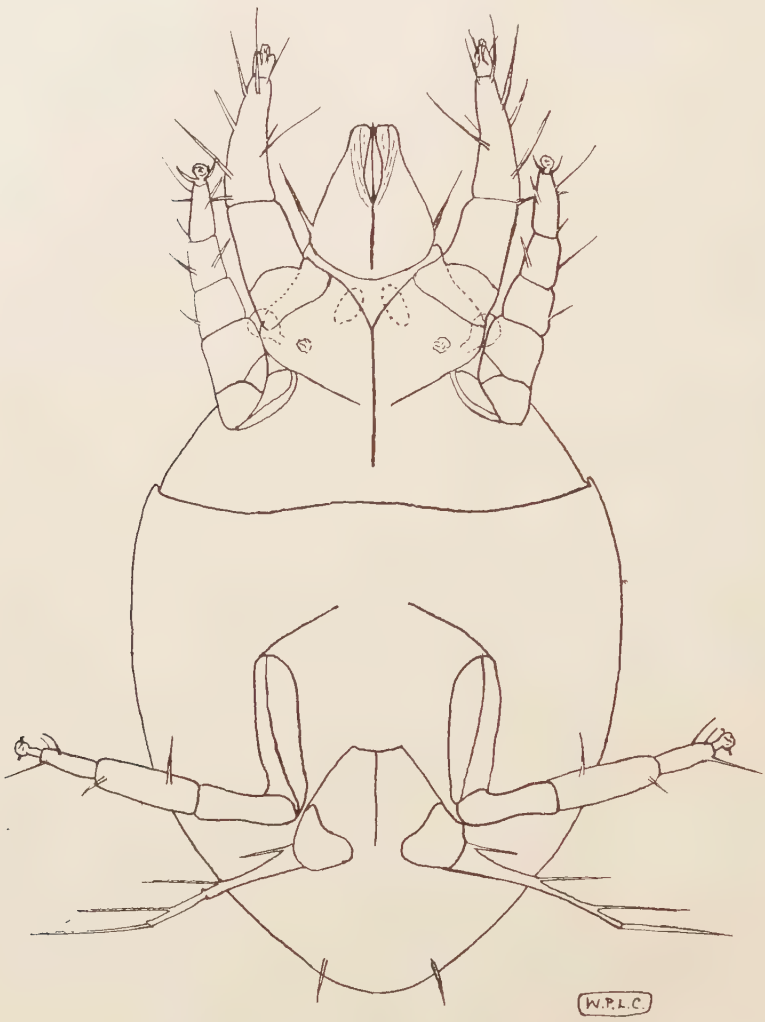

Fig. 1.

larva grows in size, these disappear more or less, giving the skin a smooth appearance. On the ventral surface the wrinkles appear, except in the anterior region of the body.

Hairs and bristles are rather rare. There are two pairs of short spines,

one pair behind the other, above the hind part of the abdomen dorsally, and also two pairs on the periphery of the abdomen far back—one pair of which is terminal. The legs possess a number of bristles, especially on the distal end.

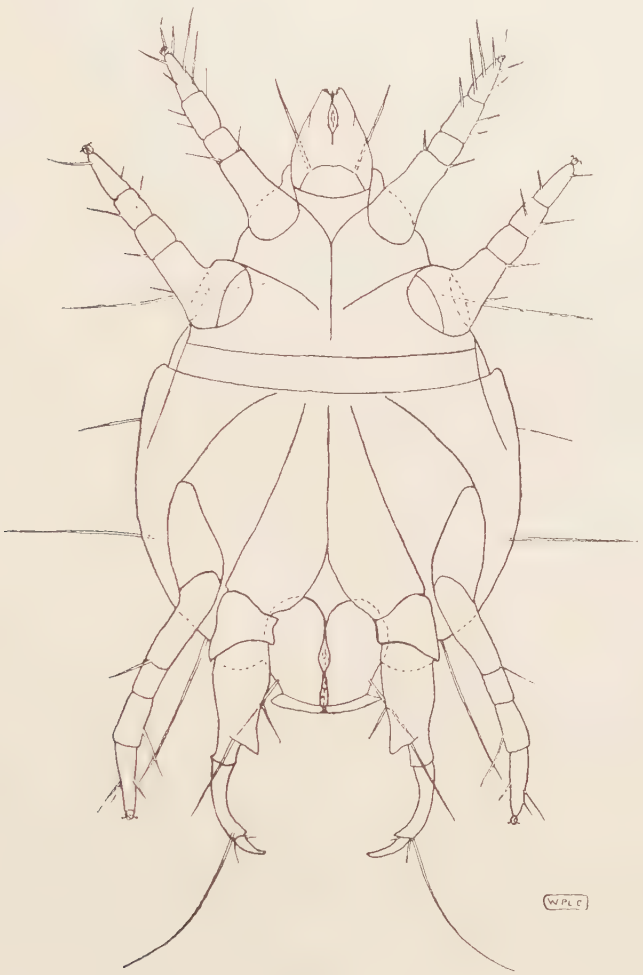

Fig. 2.

The larva of the female, when full-fed, seems to be slightly larger than that of the male. Otherwise there is no difference in appearance. The length of the larva varies from 155·25μ to 236·25μ and the breadth from 101·25μ to 128·25μ.

Quiescent Stage. There is no nymphal stage in this species and the full-fed active larva becomes quiescent, later to change into the adult

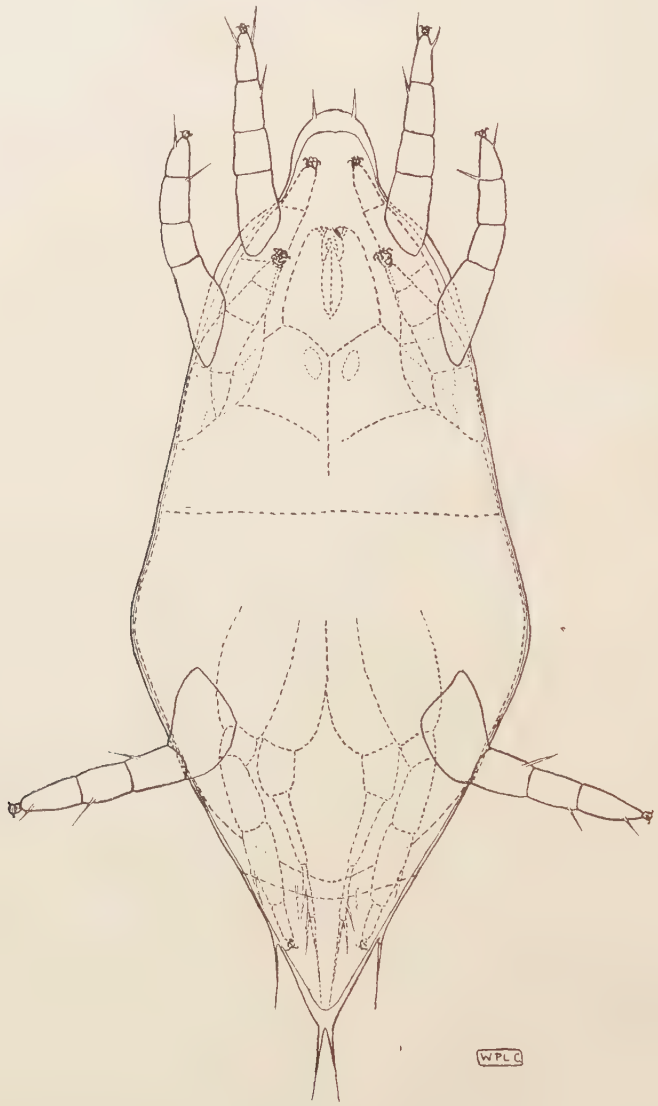

Fig. 3.

condition. This is rather unusual in mites generally. The quiescent stage (Fig. 3) is an engorged larva, which is motionless, being white in colour, slightly hyaline and without wrinkles. The new parts which appear in

the adult, such as the fourth pair of legs and the clavate organs, can be seen in formation within an apodous skin which lies inside the stretched larval skin. After a short period the larval skins split and the adult emerges.

Adult Female. It (Fig. 1) has a long oval body which is rounded posteriorly, and which has a transverse line near the junction of the cephalothorax and the abdomen. It is almost hyaline in appearance, but sometimes very dark mites have been found, this being due to ingested food. The integument is yellowish brown in colour and is somewhat chitinised. The head is rather prominent and extends backwards nearly to the middle of the epimera of the fore legs. There are two stout spines which project from the dorsal surface of the head. The mouthparts are difficult to make out but are probably similar to those of *Tarsonemus pallidus*, Banks, which, according to Ewing(14), have the palpi reduced and partially fused to the capitulum and the chelicerae needle-like.

The cephalothorax is as broad as it is long. The epimera of the first pair of legs meet at an angle in the middle line and form a narrow longitudinal furrow that extends almost to the end of the cephalothorax. The epimera of the second pair of legs are united in the same manner as those of the first pair. Between the first and second pair of legs on the dorsal aspect there is a pair of club-shaped organs, the function of which is at present unknown. The abdomen is longer and broader than the cephalothorax.

There are four pairs of legs. The two anterior pairs of legs which point forwards are equal in size and possess a number of hairs. The third and fourth pairs of legs are posterior and point backwards. Both pairs are thinner than the first and second pairs of legs. The third pair are more slender than the first and second pairs, and have the third joint rather long. The fourth pair are very thin, and show a reduced number of segments. The first pair of legs have only one claw, but on the second and third pairs there are two claws, while leg IV ends in two appendages, both of which are needle-like, the terminal one being one-and-a-half times the length of the other.

The dorsal surface is covered by shields which overlap. There are two short stout bristles above the posterior end of the body, directed backwards.

The female measures about 229μ in length, while in breadth it is about 148·5μ.

Adult Male. The colour of the male (Fig. 2) is yellow, and the body is suboval and is much smaller than that of the female. The body is widest

in the region of the third pair of legs. The head is fairly large, with one pair of dorsal bristles.

The cephalothorax is as broad as it is long. The epimera of the first pair of legs are united at the middle line; this is also the case with the second pair. The median line does not quite touch the two transverse lines, which show the division between the cephalothorax and the

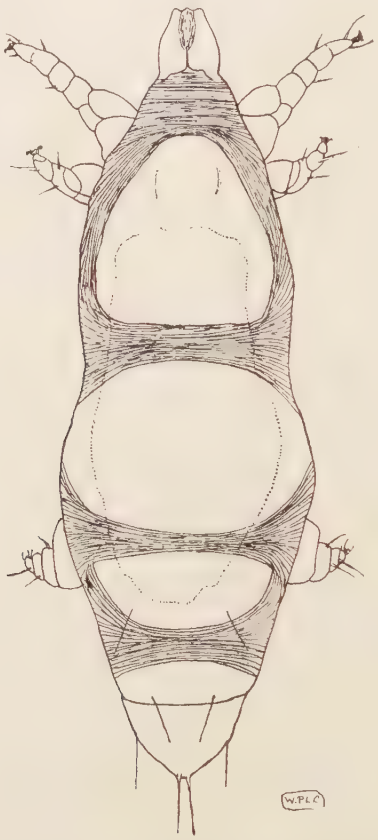

Fig. 4.

abdomen. The dorsal part of the cephalothorax is provided with one pair of exceedingly long tactile bristles.

The abdomen is larger and broader than the cephalothorax, and "its posterior part is produced backwards into an elevated process, abruptly truncated, and furnished with a pair of stout hooks at the corners. Two strong bristles proceed from the sides of the caudal process" (Warburton). There are four pairs of legs. The anterior pairs are equal in size, and possess a few moderately long bristles. The first pair have at

their apex a single claw, while the second and third pairs are two-clawed. The third pair are thinner and slightly longer than the two anterior pairs. The fourth pair of legs are very characteristic, being very stout, twice the thickness of the third pair. The first joint is broader than it is long, while the second joint is rather more than twice as long as it is broad, and is furnished on the inner surface with a broad curved expansion which has a long tactile bristle at its apex and a lateral hair near the middle. The next joint is curved inwardly, and has a small projection on its inner surface on which is mounted a short spine. Laterally, and towards the distal end of this joint, there is a very long touch bristle, which is very fine at its apex. The leg ends in a single, stout, curved, blunt hook or claw. The large epimera of the third and fourth pairs of legs, running conjointly, almost reach the transverse line.

The length of the male is about 155·25μ and the breadth about 94·5μ.

Life-history and Habits.

Oviposition. The eggs, each of which is large enough to occupy at least one-half of the female's body, are deposited one at a time at the rate of one or two per day for a variable period of from 5 to 16 days. They are found singly or in groups of from two to five, and always in moist places, such as, in the recesses formed by the curling and distortion of the pinnae, in the grooves on the upper surface of the leaf-stalk, especially near the tip of the leaf, between the fleshy scale leaves at the apex of the rhizome of the parent plant, and also of its vegetative offspring.

The eggs are placed in no set position in the folds, and they are never exposed on the plant, as they are very sensitive to the sun's rays and soon shrivel; moisture, therefore, is an essential to their well-being. When the egg hatches the shell collapses completely. The length of egg stage is from two to four days, with the temperature at 68° F. The

Table I.

Length of Egg Period.

	Date when laid	Date when hatched	No. of days		Date when laid	Date when hatched	No. of days
1.	19. v. 24	22. v. 24	3	7.	26. v. 24	30. v. 24	4
2.	20.	23.	3	8.	26.	30.	4
3.	21.	23.	2	9.	26.	30.	4
4.	23.	26.	3	10.	27.	29.	2
5.	23.	27.	4	11.	28.	31.	3
6.	25.	29.	4	12.	31.	2. vi. 24	2

length of time, however, will probably vary with the temperature. Eggs are deposited over a long period, a few being found during the winter months from November to March, but during the summer they are very numerous on the plant.

Larval Habits. When the larva emerges it ruptures the egg-shell and commences to stretch itself out. Upon hatching, nothing but the chorion is left. Later the larva crawls about on the plant, and punctures the cells, sucking up their liquid contents. It grows in size without moulting. The larval stage, varying greatly from 7 to 14 days, according to circumstances, is followed by a quiescent period, which is much shorter and less variable.

Table II.

Length of Active Larval Period.

	Date of hatching from egg	Date of becoming quiescent	No. of days		Date of hatching from egg	Date of becoming quiescent	No. of days
1.	19. v. 24	26. v. 24	7	5.	26. v. 24	1. vi. 24	7
2.	19.	26.	7	6.	27.	2.	7
3.	22.	26.	4	7.	31.	8.	8
4.	25.	8. vi. 24	14	8.	18. vi. 24	25.	7

Quiescent Stage. On being full-fed, the active six-legged larvae become motionless, usually partially attached to the plant by means of their fore legs, and possibly by their mouthparts; they are generally found in grooves or hollows among the eggs and larvae. In this resting stage development is going on and an eight-legged adult, of either sex, is being formed within an apodous skin inside the six-legged larval skin. The sexes can be determined by the form of the fourth pair of legs. The length of the quiescent period varies slightly, and the adult emerges after from one to four days. This resting stage within the old larval skin is a feature, reminiscent of the pupal or resting period of certain insects, notably that of Thrips and the coarctate pupa of Diptera (Cyclorrhapha). The writer has observed "males carrying along behind them, clasped by the grasping posterior legs, and supported on the apex of the abdomen, the six-legged, whitish, smooth, immobile body of one of these quiescent forms, whose long axis is placed at right-angles to its own." This phenomenon was observed by Moznette(22) in the case of *Tarsonemus pallidus*, Banks, on cyclamen plants. It is not certain if fertilisation takes place when the female is in the resting stage or whether the pair copulate as soon as the adult female emerges. Males have been seen to fight for the possession of one of these inert forms. Through lack of time no experiments, so far, have been done to prove this point.

Table III.

Length of Inactive Larval Period.

	Date of becoming quiescent	Date of emergence as adult	No. of days		Date of becoming quiescent	Date of emergence as adult	No. of days
1.	26. v. 24	27. v. 24	1	5.	2. vi. 24	5. vi. 24	3
2.	26.	27.	1	6.	8.	12.	4
3.	26.	28.	2	7.	8.	11.	3
4.	1. vi. 24	3. vi. 24	2				

Habits of the Adults. A short period elapses after the emergence of the adult from the resting stage, before eggs are laid at the rate of one or two per day.

The adults may be found on the plants in the greenhouse all the year round. The males are short-lived compared with the females. During the winter the males are very rare and the number of females is rather small. In early spring the numbers of both sexes increase very rapidly. From May till July the males and females are very numerous and multiplication of the species is very rapid. This is the time when most of the damage is done to the plant. As the winter approaches the numbers of the adults decrease greatly, especially in the case of the males.

The annual generations are very numerous, and these overlap completely. The complete life-cycle from egg-laying to egg-laying takes from 11 to 23 days. These figures vary greatly with the temperature and other conditions.

The adults have lived in the cells for a period of from four to five days in the case of the male and from 7 to 16 days in the case of the female. Again, these figures vary greatly.

Parthenogenesis. Tarsonemus tepidariorum seems to exhibit this phenomenon, at certain seasons of the year, especially during the winter, and very early spring. This fact is based merely on observation, since during that period males were exceedingly scarce, and eggs were found along with the females. These eggs, when put in cells, developed into females. The experiments which were set up to prove this have not yet had time to yield results.

A few quiescent larvae were put in a cell in June and after a few days female adults emerged. These females, which had not been in contact with males, lived in the cells for a period of from 6 to 16 days, but did not produce eggs. In summer, therefore, it seems that parthenogenesis does not occur.

Garman(16), working with *Tarsonemus pallidus*, found that that species exhibited the phenomenon and that the unfertilised eggs all produced females.

Hibernation. This mite evidently passes the winter mainly as an adult female. Eggs and larvae may be found at that time, but the latter are very rare.

Table IV.

Length of Adult Life.

	Date of birth of adult	Date of death	Sex	No. of days
1.	27. v. 24	4. vi. 24	Female	7
2.	28.	13.	„	16
3.	10. vi. 24	15.	Male	5
4.	11.	16.	„	5
5.	11.	19.	Female	8
6.	14.	21.	„	7
7.	17.	21.	Male	4

Nature of Injury to the Plants.

With their styliform mouthparts, the mites puncture the tissues of the plant, and suck out the juices from the injured part. The result of this is that minute depressions are formed, which later turn brown, giving the leaf a speckled appearance when the attack has been extensive. If the part has been very badly injured it turns brown and dies (Pl. V, fig. 10). Owing to the injury and continued growth about the damaged areas, the pinnae become distorted, the frond becomes asymmetrical (Pl. IV, fig. 9) and the plant is dwarfed and has a hard staring appearance. Often the pinna is so distorted and swollen that it becomes gall-like. With this reduction and disarrangement of the leaf tissues of the plant the vital processes of assimilation and respiration are checked, with the result that the plant becomes weak and unhealthy. Most of this injury is done by the mites when the frond is unfolding, old fronds not being attacked at all. Healthy plants were infected by the writer, and these plants are already beginning to show the results of mite injury.

The ultimate result of the attack is that the ferns are absolutely unsaleable (Pl. IV, fig. 8).

Migration and Spread of the Mites.

The spread of the fern mite is no doubt effected by the movement of infested fern plants from one greenhouse to another and from one nursery to another *via* the market especially.

During the winter and very early spring the plant gets an opportunity to produce fronds, which become full grown without much, if any, noticeable injury. These plants are marketed, and later they are sure to produce distorted and dwarfed foliage.

The mites migrate from one plant to another by crawling from one frond to another on a neighbouring plant when these parts are in contact. Since the mites live among the leaves of the young vegetative plants, and if these "pips" or young plants, taken from infested parents, be planted they will almost certainly become infested.

Also since it has been shown that it is possible for the mites to walk along the surface of the soil, they would thus be able to reach healthy plants, and so the disease would spread.

Healthy plants might also become infested through being potted up with infected soil.

Methods of Rearing.

After numerous unsuccessful attempts to breed the mites under observation, the following method proved successful. Two vulcanite rings, about 1 inch in diameter and about $\frac{1}{8}$ of an inch deep, were taken, and to each was fixed a circular coverglass by means of Canada Balsam. After the balsam had hardened the two rings were closely apposed,

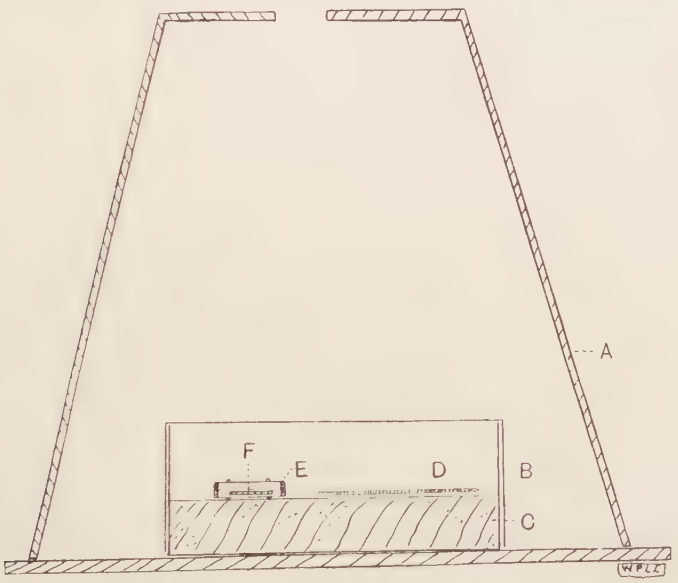

Fig. 5. A=inverted flower pot; B=petri dish; C=moist earth; D=thermometer; E=cell; F=piece of leaf.

enclosing a flat piece of young fern leaf, and fastened together by means of a copper-wire clip. This cell (Fig. 5) was placed in a petri dish, in which was also put a thermometer, and some moist earth to prevent too rapid evaporation of moisture. The petri dish was kept in semi-darkness under a large upturned flower pot.

Farrant's Medium proved to be the best for mounting specimens of *Tarsonemus tepidariorum*. The mites are placed straight into this medium without special preparatory treatment.

Other Species of the Genus *Tarsonemus*.

The majority of the species of this genus are of distinct economic importance, and are the cause of a number of diseases of plants, especially in hot-houses. The genus is one in which the life-histories and habits of the various species have been little investigated, and so little is known of these mites generally.

T. pallidus, Banks (3, 16, 22), has been found as a serious pest on cyclamen, chrysanthemum, geranium, verbena, snapdragon (antirrhinum spp.), larkspur, heliotrope, in U.S.A. and Canada.

T. floricolus, Canestrini and Fanzago(12), has been found on leaves of a large variety of plants.

T. spirifex, Marchal(21), has been reported on oats as causing elongate swellings, in Sweden, Denmark, Germany, Holland, Russia and Czecko-Slovakia.

T. translucens, Green(7), "the Yellow Tea Mite," attacks tea in Ceylon, and tea and cinchona in Java.

T. spinipes, Hirst(17), injures sugar cane in the West Indies (Barbados and Porto Rico).

T. latus, Banks(6), causes galls on the main shoots of mango, and attacks citrus fruits in Cuba.

T. ananas, Tryon(30), is reported as a forerunner of a disease of pineapples, known as "fruitlet core-rot" in Queensland.

T. chironiae, Warburton(31), has been found attacking a greenhouse plant, *Chironia exigera*, in England.

T. culmicolus, Reuter(24), causes diseases of grasses in Finland and Germany.

T. waitei, Banks(4), destroys the terminal buds of peach in New Jersey, U.S.

T. fragariae, Zimm.(9, 25, 32), attacks strawberries in Norway, Sweden, Denmark and Germany; also begonia, pelargonium and strawberry in Finland.

T. oryzae, Targioni-Tozetti(29), was found in diseased rice in Italy.

T. bancrofti, Michael, caused serious damage to sugar cane in Queensland, Hawaii and Barbados.

T. canestrinii, Marchal, produces small, rounded galls on several European grasses, *e.g. Triticum repens*.

T. approximatus, Banks(5), is reported from California on citrus trees.

T. assimilis, Banks(5), also reported from California on citrus trees.

T. kirchneri, Kramer(20), was found inside the galls of Eriophyes, and on heliotrope in Holland.

T. buxi, Canestrini and Berlese(10), was found on box leaves.

T. minusculus, Canestrini and Fanzago, was found on the body of another mite.

T. fennicum, Oudemans(23), is reported from Finland.
T. ulei is reported from Brazil.
T. chionaspivorus, Ewing(13), in U.S.A.
T. soricicola, Oudemans(23), male only was found on a shrew in Germany.
T. brevipes, Sicher and Leonardi(26), is found along with *T. floricolus* sometimes. Also attacks tobacco.
T. contubernalis, Reuter(25).
T. equipes, Sicher and Leonardi(27).
T. hominis, Dahl(8), was found on man in France.
T. intectus, Karpelles(19), occurs on barley in Hungary and also attacks man.
T. macronychus, Sicher and Leonardi(28).
T. uncinatus, Flemming(15).
T. sp.(1) was found on barley in Japan.
T. phyllophorus, Ewing, found on *Phyllostachys bambusoides*.
T. woodi (now *Acarapis woodi*, Rennie)(14, 18) causes "Isle of Wight Disease" of bees in England.

I have found mites of the genus *Tarsonemus* on begonia, bouvardia and cyclamen in nurseries near London. Tarsonemid mites attack a large variety of horticultural plants, especially Acanthaceous and Gesneraceous plants.

Suggestions for the Control of *Tarsonemus tepidariorum*.

Since mites, as a class, have a primitive method of respiration, the ordinary fumigants, such as hydrogen cyanide and carbon bisulphide, are powerless in controlling them. They are very tenacious of life under ordinary moist conditions. Sprays of all sorts have been tried in the case of other related mites, but few have met with much success. It has been known for some time that sulphur is a specific against mites, so experiments were started using this as a spray and as a dust.

A commercial preparation of colloidal sulphur, about 2 per cent., with soft soap (30 per cent.), to act as a spreader and fixative, was diluted with water at the rate of 1 in 100, and a batch of 112 plants was watered and sprayed with this solution. The fronds of the fern were unfortunately stained with sulphur and so were unsuitable for market purposes. After this set-back, the plants were watered only with the solution, but this failed to control the disease.

A second solution was made up, which was the same as the first, except that 10 per cent. of Dichlorbenzene was added. Another batch of 112 plants was watered and sprayed with this; the result did not justify the inclusion of this pungent-smelling, volatile liquid.

Ammonium polysulphide was tried as a spray, but this killed most of the plants.

Green sulphur was dusted on the plants, also proving too drastic for them. There is one chemical compound, namely pyridine, which is very deadly to most mites, and which, according to experiments conducted by Professor Lefroy, kills them at the rate of 0·0002 per cent. in water. Experiments with this liquid ought to be continued. Time has so far not permitted of this being done.

It, therefore, seems, up to the present, as if chemical treatment of the infested plants is futile.

The best method of controlling these mites seems to be to prevent the "pips" or young plants from becoming infested, and so start with clean plants. Treatment of the plants and "pips" with heat might be tried, but it is very probable that they would not stand that.

Plants, which are very badly infested, should be burned. The soil in the greenhouses, the pots, and the greenhouses themselves should all be sterilised before new plants are introduced. It would be advisable to rotate the greenhouse crops as much as possible and put in another kind of plant which will not be affected.

In areas which have been badly infested by the mites, one occasionally notices one or two plants which are either free from attack or are only slightly attacked. It might, therefore, be possible to collect these more or less immune plants, and only plant the "pips" which they produce. These plants are probably more robust, and so more able to resist the attacks of the pest. It is, therefore, an essential point to produce plants, which are as strong and healthy as possible. To attain this condition various factors play an important part.

These factors, such as proper ventilation of the greenhouses, the correct degree of temperature and humidity of the atmosphere, and adequate drainage, are important. It is also advisable to keep the plants as free from weeds as possible, and so give the ferns the best chance possible of being healthy.

Then, a matter of great importance in all probability is the kind and the proportion of the manures which are supplied to the plant. Too great an amount of nitrogen in the manure is apt to produce an abundance of luxuriant green foliage, which is rather rank, and has not the same resistance to insect and fungous diseases as has the foliage produced under the best conditions. Therefore, nitrogenous manures should be used with caution.

It has been shown by other writers (Andrews (2)), working on insect attacks on plants, that the relation of the available potash to the available phosphoric acid in the soil is important, and that, when the amount

of available potash in the soil is increased, the effect is that plants are less susceptible to injury by pests.

Lime is also supposed to give good results when added to the soil. The direct plan of campaign for the control of this pest is to produce healthy plants, and to get young "pips," which, when treated with some insecticide, will be free from mites. These plants can be kept clean by means of certain deterrents, such as sulphur and soap, and possibly pyridine and nicotine.

Since nurserymen, at present, only propagate these ferns vegetatively, it might be possible to alter this method of reproduction, when the disease is rampant, and grow the ferns from spores, which are probably fertile. If this could be done, the plants would start clean, and the chances of them growing up clean and healthy would be very bright.

These are suggestions which nurserymen might like to try and which the writer, if time permits, will investigate further.

Summary and Conclusion.

Fern mite (*Tarsonemus tepidariorum*, Warburton) is of distinct economic importance. It attacks the younger parts only of the fern (*Asplenium bulbiferum* and its varieties), causing the fronds to become distorted and the plant to be dwarfed so that it is unsaleable. The disease spreads rapidly in a greenhouse due to the rapid multiplication of the mite and the very short life-cycle of 17 days on the average. All stages of the pest may be found during the summer, and the winter is passed mainly as an adult female, although a few eggs may also be found at that time. The mites spread from one nursery to another by the introduction of infested plants; from one plant to another by crawling from leaf to leaf where these touch, and probably by walking along the surface of the moist earth; and by the planting of young "pips" taken from infested parents. The disease is difficult to control.

Preventive measures offer more chance of success than do remedial. The young "pips" should be planted clean, and then kept free from the pest by means of deterrent sprays. The "pips" are treated with some insecticide before planting.

Badly infested plants should be burnt and the soil and houses should be sterilised.

It is essential to produce healthy plants. To obtain this, certain factors are important, such as proper ventilation, adequate drainage and the correct degree of temperature and humidity.

Potash manures and lime are good. Nitrogenous manures should be used sparingly.

Rotation of the greenhouse crops is advisable.

Plants would be produced clean, if they could be propagated from spores.

I take the opportunity here of expressing my sincere thanks to Prof. Lefroy for his help and suggestions throughout the work, and to Mr Hirst for kindly identifying the mite.

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EXPLANATION OF PLATES III, IV AND V

Plate III.

Fig. 6. *Asplenium bulbiferum.* Healthy plant.

Fig. 7. *Asplenium bulbiferum*, var. *inequale.* Healthy plants.

Plate IV.

Fig. 8. *Asplenium bulbiferum.* Badly infested plant.

Fig. 9. Fern fronds, showing damage due to mite attack. (Uninjured frond on the left.)

Plate V.

Fig. 10. Old fern fronds, showing distortion and shrivelling.

(*Received September 15th*, 1924.)

Fig. 6.

Fig. 7.

CAMERON.—The Fern Mite (pp. 93—112.)

Fig. 8.

Fig. 9.

Fig. 10.

CAMERON.—The Fern Mite. (pp. 93—112.)

A DISEASE OF WILD WHITE CLOVER CAUSED BY THE EELWORM[1], *TYLENCHUS DIPSACI* (KÜHN) BASTIAN

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(With Plate VI.)

It has been known for many years that the eelworm, *Tylenchus dipsaci*, the cause of Stem Disease, can work such havoc in a crop of red clover as to render it unprofitable, and that it can infest other crops, but no description has ever been published of the effect of attacks by this eelworm on wild white clover (*Trifolium repens*)[2]. Owing to the economic importance of this plant not only in grassland for grazing, but more especially as a seed crop either in old permanent pasture or on arable, it seems desirable to describe the eelworm disease in some detail.

Occurrence.

In the course of observations and experiments on wild white clover, carried out at Wye, separate plants were grown in pots in the open, and on arable land for a number of years. During the summer of 1921 the disease was noticed amongst the pot plants and subsequently on experimental plots situated on loam, sand, and clay soils. The attack on some of the individuals under observation was so severe that the plants were hypertrophied or killed, and at a later date, a small seed trial plot was entirely destroyed by the eelworm. In addition, the disease was found (1923) in old permanent pasture in the famous grazing land of Romney Marsh, and (1924) in a field of wild white clover for seed near Biddenden, Kent.

In the latter case the clover had only been sown five years. The previous summer the crop had been dense with a good yield of seed, but when inspected the clover was stunted and scanty except in one

[1] The author is indebted to Dr T. Goodey for the following information with regard to nomenclature of this eelworm: *Tylenchus dipsaci* (Kühn 1857) Bastian 1865, syn. *T. devastatrix* (Kühn 1869) Oerley 1880.

[2] Its occurrence on this host has been recorded by Mr F. V. Theobald, *Ministry of Agriculture Misc. Publication*, No. 39, "Insect Pests of Crops," 1920–21, p. 26; and by Dr T. Goodey, *Journ. Agric. Science*, XII, Pt 1, p. 20, Jan. 1922.

corner of the field. Its dying out was thought by the grower to be due to the deseeding of the previous summer, although the next field, with precisely the same history, was in excellent condition with every promise of a heavy seed crop.

Description.

White clover, being a prostrate plant, with no upright stems, does not exhibit quite the same symptoms as those seen in red clover, and described by Amos (1) and Goodey (3). During the flowering period some or all of the flower stalks of an attacked plant may be much swollen immediately below the capitulum (Pl. VI, fig. 1), and the stems, or "runners," are found in various stages of decomposition. Those plants only recently infested require closer examination to detect the presence of eelworms, but after some practice the external symptoms are easily recognised.

The runners are short with numerous very stunted swollen branches arising in groups, the whole presenting a clubbed appearance. The leaves are smaller than usual and the petioles very short. Occasionally normal leaves are found amongst the others and both sorts are numerous, giving to the plant an appearance of being well covered with a short mat of foliage. The petioles are frequently swollen and the epidermis crinkled, though occasionally no swelling and only stunted growth is found. In more advanced stages of attack the runners become very bare, but groups of short clubbed branches with minute leaves are found distributed along their length. The runners become very fragile, and finally rot under a mass of decomposing leaves and petioles. In dry weather they turn brown and become brittle. Many plants are killed outright, but some may last for six months or a year before being killed completely (Pl. VI, fig. 2). During the attack, such plants might be well described as "stemmy" or "dying out," and although this disease has in all probability never been noticed by farmers, it seems likely that it has frequently passed unrecognised in leys or permanent pasture, its deadly effect being perhaps ascribed to "lack of permanence" of the strain of white clover affected[1].

Dissection of swollen petioles and branches revealed numerous eggs and eelworms identified as *Tylenchus dipsaci* (Kühn) Bastian[2].

[1] Other diseases found at Wye affecting wild white clover are as follows: Leaf-spots (*Pseudopeziza Trifolii*, *Polythrincium Trifolii*, *Sphaerulina Trifolii*, and bacteria not named). Rust on leaves and petioles (*Uromyces Trifolii repentis*). Leaf and Stem-Rot, with which were associated *Cephalosporium* sp. and *Fusarium* sp. Root Rot (*Thielavia basicola*).

[2] The author desires to express his thanks to Mr F. V. Theobald and to Dr T. Goodey for examining the material submitted to them.

Experimental.

The relative susceptibility of strains of white and red clover to eelworm disease was investigated, both on account of the economic importance of the white clover seed crop, and of the possibility that wild white clover, so common on the edge of most arable land, might act as a means of perpetuating the eelworm disease in red clover or other crops; the investigation was made to include a test of the susceptibility shown by various other leguminous plants.

In 1922 and 1923 infested soil was obtained by turning out those pot-plants which had been killed by the disease. The soil was heaped up and thoroughly mixed by turning over with a spade. After standing for a few days, this infected soil was again thoroughly turned over and was then used to fill the pots in which the tests were to be made. The soil of each pot was lightly pressed down and divided into two equal areas by inserting a glass partition not deeper than half an inch in the soil and reaching from one side of the pot to the other. The partition was found especially useful in preventing any of the seeds of both kinds from being washed together and consequently mixed during the necessary watering operations, and in later stages of growth of the plants it served the purpose of keeping quite distinct the two kinds of plants undergoing test, and so facilitated the counts which were made at the termination of the experiments.

On the 23rd June, 1922, the various leguminous seeds were sown, one kind on one half of each of eighteen pots, and on the remaining half areas wild white clover seed derived from a genuine old pasture was sown as the control. On the 31st May, 1923, the experiment was continued, 29 pots being sown and the same methods employed as in the previous year.

Inspection of the young plants from time to time showed that white clover was becoming infected to a greater extent than was any other kind of plant in the pots. This observation was easily made by recognition of the external symptoms. On the 9th August, 1922, and 20th August, 1923, the first of the pots were turned out and the seedlings on each side of the glass partition were counted and sorted into the following groups:

(1) Those showing unmistakable symptoms of eelworm disease, *e.g.* great swelling of one or more petioles with crinkling of the epidermis (Pl. VI, fig. 3), swelling of the "crown" and, in some cases, swelling and crinkling of the midribs of leaflets.

(2) Those which were perfectly healthy.

(3) Those which showed doubtful symptoms.

The last-named group, usually consisting of only a few specimens, was next examined. Each plant was dissected on a glass slide and presence or absence of parasitic eelworms confirmed by means of the microscope. When this confirmation had been obtained, the total numbers of diseased or healthy seedlings respectively were added to those contained in Group 1 or 2 above.

Results.

The results of the counts are recorded in Table I, where the percentage infection in each case is also stated. Susceptibility of the various kinds of plants, as compared with that shown by wild white clover, could be judged, but more exact relative susceptibility was not estimated because of the difficulty of arriving at some expression for the intensity of infection in each seedling and because the same number of viable seeds had not been originally sown in each case.

With reference to the 1922 experiments, cultivated strains of white clover and even once grown wild white, proved particularly susceptible, an average of 60·7 per cent. of the seedlings being attacked. The resistance shown by red clover strains was remarkable, an average of only 1·7 per cent. being affected. The comparatively small variation in the percentage of control plants attacked in each pot showed that by mixing the soil thoroughly, even distribution of the parasites had been secured. Of the 1923 experiments the same cannot be said. Both the control and tested plants remained unaffected in 19 of the 29 pots and it was found that the majority were attacked by the fungus, *Thielavia basicola*, causing severe root rot[1]. It was thought possible that this may have hindered infection by the eelworm and may account for the differences in intensity of attack in the controls, which in the previous year were much more evenly affected by the eelworm. The remaining ten pots were examined and the counts are recorded in Table I.

The results arrived at are very different from those obtained by Amos(1)[2] and by Goodey(3), who used for their infective material eelworms from diseased red clover. Although there is no mention by the latter author that one strain of clover was used throughout on all pots

[1] *Thielavia basicola* was found attacking sainfoin, red clover, wild red clover, cultivated white clover, wild white clover, alsike clover, trefoil (*Medicago lupulina*), and trifolium (*Trifolium incarnatum*).

[2] Amos(1) states: "White clover (Dutch variety) is but slightly susceptible....I have never found white clover, sown pure, attacked by this disease."

Prof. D. A. Gilchrist(2) also reports that at Cockle Park, "Ordinary red clover and white clover die off very quickly there, but wild white clover retains its permanent character, and eelworm attack has not been found on this plant at that station."

Table I.

Susceptibility shown by Strains of White Clover and other Forage Plants to Tylenchus dipsaci *obtained from Wild White Clover.*

Pot No.	Plant	1922 Total tested	1922 No. healthy	1922 No. infected	1922 % infected	1923 Total tested	1923 No. healthy	1923 No. infected	1923 % infected
1.	Bokhara clover	210	170	40	19	—	—	—	—
	Control	217	160	57	26	—	—	—	—
2.	Sainfoin	33	33	0	0	69	69	0	0
	Control	95	76	19	20	124	108	16	13
3.	Perennialised broad red clover	246	231	15	6	280	280	0	0
	Control	174	152	22	13	105	101	4	4
4.	English broad red clover	282	280	2	1	—	—	—	—
	Control	151	123	28	19	—	—	—	—
5.	Chilian mammoth red clover	240	239	1	½	—	—	—	—
	Control	124	95	29	23	—	—	—	—
6.	Trifolium, English late red	31	31	0	0	—	—	—	—
	Control	278	253	25	9	—	—	—	—
7.	White clover, Danish, Stryno	220	51	169	77	187	184	3	2
	Control	229	150	79	34	148	148	0	0
8.	Yellow suckling clover	21	20	1	5	—	—	—	—
	Control	123	101	22	18	—	—	—	—
9.	White clover, Danish, Morso	163	45	118	72	—	—	—	—
	Control	123	100	23	19	—	—	—	—
10.	Trefoil	5	5	0	0	—	—	—	—
	Control	61	44	17	28	—	—	—	—
11.	Single-cut cowgrass	144	141	3	2	201	201	0	0
	Control	100	72	28	28	147	93	54	37
12.	Red clover, Danish, Hersnap	263	263	0	0	—	—	—	—
	Control	119	95	24	20	—	—	—	—
13.	Lucerne	105	93	12	11	197	197	0	0
	Control	161	114	47	29	122	100	22	18
14.	English giant hybrid cowgrass	166	164	2	1	—	—	—	—
	Control	142	108	34	24	—	—	—	—
15.	English perennial white clover	174	36	138	79	—	—	—	—
	Control	152	114	38	25	—	—	—	—
16.	American wild (?) white	159	39	120	75	—	—	—	—
	Control	178	147	31	17	—	—	—	—
17.	Ladino mammoth white clover	174	167	7	4	—	—	—	—
	Control	119	79	40	34	—	—	—	—
18.	Wild white, once grown	146	69	77	53	—	—	—	—
	Control	154	121	33	21	—	—	—	—
19.	White Dutch, Gröningen	—	—	—	—	166	161	5	3
	Control	—	—	—	—	135	134	1	1
20.	White Dutch, Friesland	—	—	—	—	163	137	26	16
	Control	—	—	—	—	114	112	2	2
21.	Alsike clover	—	—	—	—	337	334	3	1
	Control	—	—	—	—	147	90	57	39
22.	Wild red clover	—	—	—	—	161	161	0	0
	Control	—	—	—	—	210	200	10	5
23.	Red clover, Welsh	—	—	—	—	219	219	0	0
	Control	—	—	—	—	204	190	14	7
	Six red clovers (1922)	1341	1318	23	1·7				
	Six white clovers (1922)	1036	407	629	60·7				
	All controls (1922)	2700	2104	596	22·1				

as a control, it is evident that in that experiment, and in those of Amos red clovers were more susceptible than the white.

This disparity between results of similar experiments may be accounted for by the possibility of there being biologic forms of eelworms, or forms having a particular taste or preference for one kind of plant which they have infested for several generations[1], and if this should prove to be the case, relative susceptibility to eelworm attack shown by various leguminous plants would depend as much upon the preference shown by the particular form of eelworm present as upon any faculty of resistance possessed by the plants.

As a result of observation of the disease in the field, it is evident that the modern method of growing wild white clover in a nearly pure state on arable land renders that valuable crop more liable to an epidemic of eelworm disease than under the original system on old pasture, where the individual plants are to some extent separated by grasses. In cases where white clover is attacked at all, it seems highly probable that cultivated white would be the first to die out as a result of the disease. The question arises as to how many failures to keep Dutch white clover in long leys may have been due to this eelworm.

Summary.

A disease of white clover, due to the eelworm, *Tylenchus dipsaci*, has been found to cause serious harm. This and other diseases are thought to be partly responsible for the lack of permanence sometimes ascribed to strains of white clover. In tests of the susceptibility shown by various clovers to the attacks of the eelworm from wild white clover, remarkable resistance was shown by red clovers and the maximum susceptibility by cultivated white clovers. The suggestion is made that in future tests of the relative susceptibility of various plants to attack by this parasite, the former hosts of the eelworm should be taken into account.

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[1] This theory was put forward by Dr J. Ritzema Bos(5). "It must also be added that eelworms of which the progenitors have developed for a considerable number of generations in the same plant, are not easily transferred to another kind of plant, or at any rate do not multiply vigorously there." A similar observation was subsequently made by Miss Ormerod(4).

Fig. 1.

Fig. 2.

Fig. 3.

WARE.—A Disease of Wild White Clover caused by the Eelworm. (pp. 113—119.)

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EXPLANATION OF PLATE VI

Fig. 1. "Runner" of wild white clover affected with eelworm disease. Dense clusters of decaying leaves (*right*) and swelling of leaf petiole and flower stalk below the capitulum (*left*) are features of diagnostic value. The main stem is beginning to decay. (Natural size.)

Fig. 2. Pot plants 24 and 38, the latter showing the effects of eelworm disease. Plant 38 was formerly as vigorous as plant 24 on the left. (One-ninth natural size.)

Fig. 3. White clover seedling, showing characteristic effect of eelworm disease. The petiole is enlarged and epidermis wrinkled. (Two-thirds natural size.)

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